Drug handling in fit and frail elderly people

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DRUG HANDLING IN FIT & FRAIL ELDERLY PEOPLE

Submitted by Susan E Ellmers

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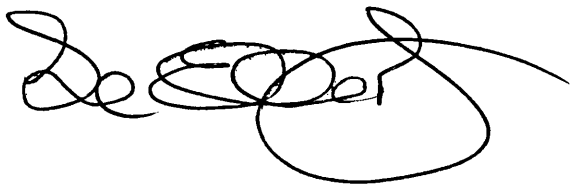

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DEDICATION

To my dogs

A faithful friend is the medicine of life.

(Ecclesiasticus vi, 16)

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I would like to acknowledge the importance to me of the following individuals and organisations, who have each in their own way contributed to the completion of this thesis.

My family who are always there; Dr. Luke Parker, without whose practical help and support life would have been much harder; Dr. Lidia Notarianni, who has my sincere thanks for her encouragement and guidance; Dr. Chris Lovell, whose expertise in eczema management has seen me through some bad times, and whose advice has shown me the way forward; Dr. Roy Jones and the staff at the Research Institute for the Care of the Elderly, for their help and use of the excellent HPLC; Staff at St. Martin's Hospital, for their friendly co-operation; The General Practitioners at St. Mark's Road Surgery, Bath, who allowed me to enlist the help of their staff, computer and patients to carry out much of this work; The University of Bath, for providing financial support, and the School of Pharmacy & Pharmacology for the use of its facilities. I am also grateful to Gustav Mahler (1860-1911) whose music helped me through the long nights. I would finally like to thank all those volunteers who took part in this study, without whose participation and co-operation none of this would have been possible.

ABBREVIATIONS

AUC	area under the time v plasma concentration curve
CCr	creatinine clearance (ml/min)
CCram	creatinine clearance from morning urine collection (ml/min)
CCrn	creatinine clearance from overnight urine collection (ml/min)
CCrpm	creatinine clearance from afternoon + evening urine collection (ml/min)
CCr/SA	creatinine clearance normalized to $1.73m^2$ body surface area ($ml/min/1.73m^2$)
CCr0-6	creatinine clearance 0 - 6h postdose (ml/min)
dCCrn	difference between measured 24h CCr & CCrn = CCr - CCrn
%CCrn	percent difference between CCr & predicted CCr = CCr / CCrn x 100
Cl	apparent clearance
Cl/kg	apparent clearance per kg bodyweight
Clr	renal clearance
Clr/kg	renal clearance per kilogram bodyweight
Clr0-6	renal clearance 0 - 6h postdose
Cls	apparent serum clearance
Cls/kg	apparent serum clearance per kilogram bodyweight
DIG	digoxin
%Du	percent of dose administered recovered in urine
FM	Frumil tablets
FRU	frusemide
FS	frusemide BP tablets
HPLC	high performance liquid chromatography
M.Sc	mobility score
PAR	paracetamol
PARG	paracetamol glucuronide
PARS	paracetamol sulphate
Q1 & Q3	lower (25%) & upper (75%) quartiles

ABBREVIATIONS (contd)

RIA	radioimmunoassay
SA	body surface area
SCr	serum creatinine concentration (mg/100ml)
s.d.	standard deviation
[SDIG]	serum digoxin concentration
[SPAR]	serum paracetamol concentration
t1/2	elimination half-life
UCr	urinary creatinine (mg)
[UCr]	urinary creatinine concentration (mg/100ml)
free UPAR	urinary paracetamol
UPARG+S	urinary paracetamol glucuronide + sulphate
total UPAR	total urinary paracetamol (UPAR + UPARG+S)
Vd	volume of distribution

MEDICAL ABBREVIATIONS

AF	atrial fibrillation
Ca	carcinoma
CABG	coronary artery bypass graft
CCF	congestive cardiac failure
COAD	chronic obstructive airways disease
CVA	cerebrovascular accident
DVT	deep vein thrombosis
#	fracture
GU	gastric ulcer
HH	hiatus hernia
IDDM	insulin dependent diabetes mellitus
IH	inguinal hernia
IHD	ischaemic heart disease
LVF	left ventricular failure
MI	myocardial infarction
MND	motor neurone disease
MS	multiple sclerosis
NG	neoplastic growth
NIDDM	non-insulin dependent diabetes mellitus
NOF	neck of femur
OA	osteoarthritis
PE	pulmonary embolus
RA	rheumatoid arthritis
SOB	shortness of breath
THR	total hip replacement
TIA	transient ischaemic attack
TURP	trans-urethral resection of the prostate
UC	ulcerative colitis
URTI	upper respiratory tract infection
UTI	urinary tract infection

ABSTRACT

1 "The elderly" do not form a single homogeneous group, and those to whom medication is most commonly prescribed were identified as forming at least two separate groups: those who are active & able to live independently in the community, and those who are infirm & immobile, requiring help with activities of daily living. Definitions were constructed to enable these groups to be readily differentiated without laboratory investigations, using a combination of social & functional criteria. Terms "fit" & "frail" were used to describe these two populations.

2 Creatinine clearance was measured in 245 elderly people. In addition to those subjects who were defined as fit or frail, a third group of healthy elderly people were recruited from the Research Institute for the Care of the Elderly volunteer panel & categorised as "very fit". CCr, UCr & SCr were significantly different between females & males. No significant difference was found between the CCr of age-matched very fit & fit subjects, although both groups exhibited the normal reduction in CCr known to occur with increasing chronological age. Frail subjects had significantly lower CCr than their age-matched very fit & fit counterparts, suggesting that frailty imposes an additional decrement on CCr in old age.

3 CCr calculated from the usual 24h urine collection was found to be accurately & reliably predicted when urine collections of about 8h were instead employed. Time of day over which the collection was carried out appeared to have little effect on prediction accuracy, although an overnight urine collection is probably the simplest &

most convenient to carry out. Neither diuretic use, magnitude of measured CCr nor frailty influenced the accuracy of CCr prediction.

4 CCr was predicted using serum creatinine concentration in conjunction with a variety of equations available for the purpose. Accuracy of prediction was greatest when equations were employed which incorporated patients age & bodyweight, but in all cases this method was found to be less reliable than those using reduced urine collection times. Accuracy of CCr prediction by equation was not influenced by diuretic use, although those subjects with the lowest CCr, who were mostly frail, tended to have CCr overpredicted.

5 Elimination $t_{1/2}$ and serum & renal clearance of frusemide were measured and compared between groups of fit & frail elderly subjects. Those subjects taking Frusemide BP tablets cleared FRU less efficiently than those taking Frumil tablets. No significant difference was found between the mean % dose recovered in urine of the fit & frail groups. FRU $t_{1/2}$ tended to be increased & FRU clearances reduced in the frail groups. CCr was also significantly lower in the frail groups. When fit & frail subjects taking Frumil were age-matched, the rate of FRU excretion remained slightly reduced in the frail group. While CCr appears to be more important than frailty in the determination of the rate of FRU elimination, frailty is associated with a reduced CCr below that expected from age alone.

6 Paracetamol was selected as a model drug to compare the efficiency of hepatic drug clearance between fit & frail

elderly subjects. The frail group had a significantly increased elimination $t_{1/2}$, reduced serum clearance & reduced recovery of urinary PAR + metabolites. The frail group was significantly older, and so fit & frail subjects were age-matched and again compared; similar trends remained. While impaired absorption in frail subjects could explain the reduced clearances observed in this group, the increased t1/2 could not be accounted for in this way. These results suggest that hepatic drug clearance is impaired in frail subjects.

7 Serum & renal clearance of digoxin were compared between groups of fit & frail subjects, all of whom had been regularly taking DIG for a variety of cardiovascular conditions, and were known to be in steady-state. Clearance was found to be significantly reduced in frail subjects who also had a reduced CCr, while % dose recovered in urine over 24h was similar between both groups. When fit & frail subjects were matched for CCr this trend was lost. These results suggest that the additional reduction in CCr associated with frailty, rather than frailty per se, may impair the efficiency of DIG elimination in the frail elderly.

8 Results from this thesis suggest that when drugs are prescribed for frail elderly patients, dosage levels should take into account the additional decrement in renal & hepatic drug clearance which appears to be associated with this state. If frail elderly patients received reduced dosages compared to their fit counter-parts, the excess of dose-related adverse drug reactions known to occur in elderly people may be prevented.

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CHAPTER ONE

INTRODUCTION

At present about 20% of the British population are aged 60 years or over and demographic trends indicate a continual expansion in the proportion of elderly people in developed countries in the foreseeable future (1,2). This increase is predicted to be steepest in the very elderly age group, and while the UK already has 2 million people aged over 80, 12% of the population will be over 75 by the year 2020 (3,4). Disability increases with age and over 40% of retired people are limited in their activities by chronic ill health, with this figure increasing sharply for the very elderly (5). The proportion of those who are housebound increases from 1% of the population in the age group 65-69 to over 20% after 85 (6,1).

From these statistics it is not surprising that the elderly are the major consumers of prescribed medication, and figures from the British Pharmaceutical Industry for 1985 indicate that they account for 35-40% of drug expenditure (3,7). Adverse drug reactions are known to be two to three times more likely in the elderly age group due to their increased drug consumption and altered drug response.

In recognition of these patterns of drug use & misuse licensing authorities have now set down requirements for studies to be conducted on new drugs in old age. As FI Caird commented "For new drugs likely to be a risk for the elderly there are stringent requirements for testing in old age, and close monitoring. Thus what has for years been immoral & unethical has suddenly become compulsory" (8).

1.1 PHYSIOLOGICAL & PHARMACOKINETIC CHANGES OF OLD AGE

Various physiological changes associated with the normal ageing process are implicated in the excess of adverse drug reactions occurring in old age. The main changes seen even in the absence of specific pathology are listed below :-

i) decreased gastric acid secretion & reduced GI motility
ii) altered body composition
iii) decreased plasma albumin concentration
iv) decreased liver mass & hepatic blood flow
v) decreased renal blood flow, GFR & tubular secretion

These physiological changes can influence pharmacokinetic profiles via alterations in drug handling during absorption distribution, metabolism and/or excretion as given below.

Drug Absorption

Changes in function of the gastrointestinal tract in old age include a reduction in gut surface area, a decrease in gastric parietal cells leading to reduced gastric acid secretion (to elevate luminal pH), and impaired splanchnic blood flow, gastric motility & gastric emptying (9,10). Most drugs in clinical use are absorbed via passive diffusion and neither rate nor extent of absorption appears to be significantly delayed following these age-related changes (11). Studies comparing the rate & extent of absorption of paracetamol, sulphamethizole, phenylbutazone, lorazepam & aspirin have shown no difference between groups of young & elderly subjects (12,13,14). However, active transport mechanisms appear not to function as well in the elderly and the absorption of some vitamins & minerals such as iron, calcium & thiamine may be impaired (15,16).

Distribution

Body composition changes with age when the proportion of lean body mass and body water declines and adipose tissue increases (9). Studies relating muscle mass to age have shown a 30% reduction by the age of 80, and this figure is further increased by ill health & immobility (17). While this trend is seen in both sexes, elderly males tend to retain a greater proportion of muscle : adipose tissue than their female counterparts of similar age & weight (17).

The degree of plasma protein binding also determines drug distribution. Many basic drugs have a high affinity for α_1-acid glycoprotein whose concentration tends to increase in old age (18). Binding of basic drugs such as propranolol may thus increase in the elderly but this change is not considered clinically relevant. Acidic drugs have a higher affinity for plasma albumin whose concentration declines in old age (18). While this decrease is small in the healthy elderly, differences are more pronounced in the presence of chronic disease and also malnutrition, thought to affect up to 12% of those aged over 80 (19). Thus binding of acidic drugs is reduced in old age but again clinical relevance is usually not significant. However, when an increase in free fraction occurs in conjunction with pharmacokinetic changes greater clinical significance may be assumed.

Hepatic Metabolism

Metabolism by the liver is, for the majority of drugs, the rate-limiting factor in their duration of action, and many studies have been conducted in an attempt to determine whether the efficiency of hepatic metabolism is compromised in the elderly. Hepatocytes carry out a range of reactions

which contribute to the removal of drugs, traditionally termed Phase I & Phase II biotransformations. Phase I reactions include oxidation, reduction & hydrolysis which render drugs more polar. Phase II reactions involve conjugation of the drug or its Phase I metabolite(s) to a larger endogenous substituent such as glucuronide, sulphate or acetate to give a much more polar compound, facilitating excretion into urine. Whilst no clear pattern has arisen from studies into age-related changes in drug metabolism, two general trends have emerged :-

i) drugs undergoing hepatic microsomal oxidation (Phase I) are likely to be more slowly metabolised in the elderly (eg. diazepam) whilst those that are conjugated (Phase II) are usually not influenced by age (eg. lorazepam),

ii) drugs with high hepatic clearances & extraction ratios, which undergo extensive first-pass metabolism during oral dosing, may show substantially increased bioavailability in old age (20).

These trends associated with hepatic senescence could be due to either a decrease in number or activity of enzymes responsible for biotransformation, or a reduction in rate of drug delivery to the liver (impaired hepatic blood flow)

Hepatic blood flow, rather than microsomal-enzyme activity, is the major determinant of total clearance of many commonly used drugs that are collectively termed "flow limited" or "highly extracted" drugs (eg. chlormethiazole). Studies have shown conclusively that apparent hepatic blood flow declines with age, by about 35% in those over 65 years compared to those under 40 years, even after allowing for changes in body weight (21). Furthermore, liver perfusion (liver blood flow per unit of liver volume) has also been

found to fall by about 11%. The relevance of this is that drugs which are readily metabolized by the liver will exhibit an increased bioavailability after oral dosing since the degree of first-pass metabolism is reduced.

Other drugs undergo "capacity limited" metabolism when the hepatic extraction ratio is low (eg antipyrine,imipramine). Studies have failed to show a correspondence between activity or affinity of specific Phase I enzymes & chronological age in humans (19,20,21,22). However, using ultrasound it has been shown unequivocally that liver volume is negatively correlated with age, when expressed in absolute terms or in relation to body weight, and a 28% fall in liver volume has been noted in those over 65 years when compared to those aged less than 40 (21).
Thus changes in liver size may be the major factor determining decreased elimination of capacity-limited drugs with age, and any changes in enzyme activity are secondary to this, at least in the healthy elderly (21).

To date, the consensus of opinion is thus : liver size & blood flow declines with increasing age, and the reduced elimination of both capacity-limited & flow-limited drugs seen in the elderly is likely to be due to these morphological and physiological changes. These changes may at least in part explain the excess of dose-related adverse drug reactions in old age. Unfortunately there is no easy way of assessing the efficiency of an individuals hepatic drug clearance before initiating treatment with hepatically metabolised drugs. This emphasises the need for research into the change in hepatic function in old age.

Renal Excretion

The best documented alteration in pharmacokinetics with age is the reduction in rate of renal drug elimination. Between the fourth & eighth decades of life human kidneys lose approximately a fifth of their weight from a variety of morphological changes (23). Beginning in mid life, renal blood flow decreases progressively from about 600ml/min at age 40 to 300ml/min at age 85 (24). Similarly a normal GFR of 120ml/min at age 40 declines to about 60ml/min at age 85 and this closely correlates with a decline in creatinine clearance (25). Tubular mass declines in a comparable way to renal blood flow & GFR, and both tubular secretion & reabsorption are impaired in old age (24). Essentially, ageing produces impaired homoeostatic flexibility, with the kidneys less able to conserve water and salts, and also less able to handle a water or solute load (26,27).

For any drug whose clearance is accomplished entirely or partially by renal excretion of the parent drug, rate of elimination will decline in proportion to the inevitable reduction in GFR and tubular secretion observed in old age. Thus accumulation of the parent drug or renally excreted metabolites may occur if drug dosages are not altered appropriately when prescribing for elderly people. Fortunately renal function may be estimated by a variety of methods to facilitate the accurate calculation of drug dosage levels when renally cleared drugs with a narrow therapeutic window are prescribed. The accuracy with which renal function may be predicted has itself been subject to extensive research since errors in prediction could lead to inappropriate therapy.

1.2 WHO ARE THE FRAIL ELDERLY?

Even in the absence of discernable disease there is a substantial heterogeneity in the physiological abilities of old people. Some remain independent until well into their ninth decade, exhibiting little loss of function even at this advanced age, while others require full care soon after retirement. It is possible that lifestyle rather than the intrinsic ageing process underlies much of the age change in physiological processes. To date few studies have attempted to identify physiological and biochemical differences between those elderly who are generally fit and active and those who are almost entirely dependent on others in the absence of acute physical illness. Most research into drug handling in old age has concentrated on groups of "fit individuals", frequently ill defined, and implicit extrapolations have subsequently been made for frail patients (28). The fit elderly are undoubtedly an easier group to study since they are able to give valid informed consent, and are free from disease and subsequent medications which may confound the effect of old age per se. Conversely, their less fit counterparts are frequently unable to give informed consent, posing a range of ethical dilemmas before the trial outset; in addition, they will almost without exception be receiving medications which may interfere with the studies.

The first attempt to standardize the selection of subjects for studies into the effects of ageing was made when a working party was set up within the framework of the European Community EURAGE Concerted Action Programme on Ageing. The resulting document, "The Senieur Protocol", was published in 1984 to establish strict admission

criteria for studies on immunogerontology in man, based on clinical information and biochemical data, and setting limits for pharmacological interference (29). The aim was to contribute to the dissection of the influence of disease versus ageing on the immune system. However, the exclusion criteria set down to select healthy participants is extreme and impracticable for the purposes of the present set of investigations.

An alternative operational definition has been proposed by a second group in an attempt to dissociate biological and chronological age and the terms "fit" & "frail" have been coined for this purpose (28); their definitions are below:-

The fit elderly are individuals, over 65 years of age, living independently at home or in sheltered accommodation. They are freely ambulant and without significant hepatic, renal, cardiac, respiratory or metabolic disorder on either clinical examination or laboratory investigation. They do not receive regular prescribed medication.

The frail elderly are individuals, over 65 years of age, dependent on others for activities of daily living, and often in institutional care. They are not independently mobile; whilst they do not have overt cardiac, respiratory, hepatic, renal or metabolic disease minor abnormalities may be revealed on laboratory investigation. They may require regular prescribed drug therapy. Conditions contributing to frailty commonly include Alzheimer's disease, multi-infarct cerebro-vascular disease, Parkinsonism, osteoporosis, osteoarthritis, and healed fracture events.

Although the distinction proposed is based primarily on social and functional criteria it is paralleled by psychological and physiological differences. Using these

definitions differences in hepatic function between the fit & frail elderly has undergone investigation. While the fit elderly exhibit the normal biological reduction in liver volume & blood flow associated with old age the frail elderly appear to possess an additional decrement in hepatic function to further reduce hepatic drug metabolism, which may be due to a reduction in the specific activity of some hepatic enzymes (19,20,22,30). While the fit elderly similarly undergo a reduction in renal function as a normal consequence of ageing, it has yet to be determined whether an additional decrement is imposed upon the frail elderly to further reduce drug clearance beyond that expected. The frail elderly also appear to have reduced mental function, reduced total body potassium, alterations in electrolytes, and a reduced serum albumin is apparently common (28).

Distinction between apparently different groups of elderly people is obviously important in the field of gerontological research and geriatric practice. However, the definitions above appear to exclude the majority of elderly people who are usually cared for by General Practitioners, who have the appearance of "fitness" whilst taking regular medications for well controlled chronic conditions. It seems important that the differences between these elderly subjects and their less fit counterparts, who have similar chronic conditions but are nevertheless considerably more infirm, be examined. Therefore, the main aim of this thesis was to successfully identify, define and compare these groups of elderly people, in terms of renal function and hepatic & renal drug clearance.

1.3 CREATININE CLEARANCE STUDIES IN ELDERLY PEOPLE

For drugs whose clearance is accomplished entirely or partially by renal excretion of the intact drug, total clearance will predictably decline in approximate proportion to the reduced GFR. It is therefore desirable to know the GFR of a patient before such drugs are prescribed to prevent accumulation, particularly when the therapeutic window is narrow.

Creatine occurs almost exclusively in muscle where it serves to guarantee the continuous supply of energy necessary to perform work. The breakdown of creatine liberates energy and creatinine is the final metabolic product of this process. Creatinine is released into the bloodstream at an almost constant rate in a person not taking severe exercise and in the absence of active muscle damage, and subsequently renally excreted mainly by glomerular filtration, although a variable amount is actively secreted by the renal tubules (31,32).

For some time it has been recognized that knowledge of renal function would be advantageous in order to assess the degree of renal damage in certain disease states, to measure changes in renal function over time, and to aid prescribing of drugs. For GFR to be accurately measured the ideal substance should be metabolically inert and excreted exclusively by glomerular filtration, ie. neither reabsorbed nor secreted by renal tubules. In 1926 it was suggested that since creatinine is produced at a constant rate, and excretion is mainly via glomerular filtration, measurement of endogenous creatinine clearance (CCr) would provide a good estimate for GFR (33). This could be practically carried out by collecting urine for 24 hours

and comparing the quantity of creatinine excreted in urine with the concentration of creatinine in serum. Since the rate of urine production would be known, creatinine clearance could then be calculated as for any other clearance value, to give an estimate of GFR.
Since this time many studies have been carried out to compare CCr with GFR measured by other more specific methods. Serum creatinine concentration (SCr) has also been suggested to provide a good estimate of renal function with the advantage that it is easier to obtain (34,35). However, the decline in muscle mass & lean body mass in old age is often overlooked, and since SCr levels depend upon creatinine turnover as well as renal function, the reduced renal function in the elderly usually does not give a meaningful elevation in SCr (36). To many it appeared that the most helpful & easily obtained result would be if SCr was measured and then used to predict CCr by use of a mathematical formula. Various formulae have subsequently been produced, the earliest in 1959 (37), and to date 12 equations & 4 nomograms exist. Further studies have attempted to determine which, if any, formula most accurately predicts CCr in healthy individuals and in those with specific disease states. One problem addressed in this thesis is whether these formulae predict CCr equally well in both fit & frail elderly subjects. Accuracy of CCr prediction using formulae is also compared with that using a urine collection of less than 24 hours duration. If CCr can be accurately predicted, prescribing of drugs which are renally cleared may thus be made safer in this age group who are at greatest risk of adverse drug reactions.

1.4 DRUG STUDIES IN ELDERLY PEOPLE

A number of studies have been conducted to determine which groups of drugs the elderly are most frequently prescribed, and of those, which are associated with the highest incidence of adverse reactions. The most recently published study to examine drug use in patients admitted to hospital found that out of all the principal drug groups prescribed, diuretics were the most commonly prescribed drug, identified in over 40% of admissions (38). Also significant in patients' drug regimes were hypnotics & anticonvulsants (25%), analgesics & antipyretics (25%) and digitalis alkaloids (16%). For this population, adverse drug reactions were identified in 10% of subjects taking medication, and drug classes most frequently implicated were antidysrhythmics, antihypertensives, NSAIDS, diuretics and digitalis alkaloids.

The aim of this thesis was to examine the efficiency of drug elimination in elderly people and to determine whether frail subjects exhibited an altered pattern of excretion when compared to their fit counterparts. This would be most relevant if the drugs which were most commonly prescribed and frequently implicated with an excess of adverse drug reactions were investigated. When drug usage in the Bath area was examined, both in the community and hospitals, frusemide, either alone or in combination with amiloride, was the diuretic most frequently prescribed. Frusemide is eliminated mainly via renal excretion, and so it seemed appropriate to choose a second drug which is hepatically cleared to compare the two modes of drug clearance. Paracetamol was found to be the most commonly used drug known to be solely hepatically cleared. The

excess morbidity & mortality associated with digoxin use has been well documented for many years, and this seemed to be a suitable third drug for close investigation. Therefore, in addition to the studies relating to measurement & prediction of CCr in fit & frail elderly people, this thesis was extended to investigate the efficiency of excretion/metabolism of three "model" drugs, that is, frusemide & digoxin which undergo mainly renal excretion, and paracetamol whose elimination is accomplished via hepatic metabolism.

1.4.1 FRUSEMIDE

The elderly receive a disproportionate number of drugs on prescription, and diuretics are one of the most common classes prescribed - it has been estimated that one third of people aged over 65 take a diuretic (39,40,41). By definition a diuretic induces a diuresis of water & solutes with the loss of sodium essential for the anti-oedema effect. However, sodium loss is inevitably accompanied by loss of other ions, and this is a potentially serious side-effect (42). Modern diuretics have revolutionized the treatment of oedema, and removal of fluid from peritoneal & pleural cavities by aspiration, or from the limbs by the insertion of tubes, is now rarely practised (43). Although these agents are invaluable in the control of hypertension, congestive cardiac failure & other oedematous conditions, widespread use of diuretics is often questionable, and some older persons undoubtedly receive diuretics inappropriately to reduce gravitational ankle oedema (39). Thus the risk-benefit ratio should always be considered before initiating diuretic therapy, and their prescription should be regularly reviewed during chronic therapy (42).

Mode of Action and Therapeutic Uses

Frusemide (furosemide, FRU) is a member of the group of "loop" or "high-ceiling" diuretics - the others are bumetanide, piretanide & ethacrynic acid. All have a rapid onset of action, cause a brisk diuresis and have a short duration of action (32). FRU is a "loop" diuretic since it appears to inhibit specific enzymes concerned with pumping chloride ions (and therefore NaCl) across lining cells of the ascending limb of the loop of Henle. The site of action is reached intraluminally after the drug has been excreted by the proximal tubules. Potassium secretion into the distal convoluted tubule is increased because of the exchange of potassium for sodium, under the influence of aldosterone and increased intraluminal sodium, and this leads to increased potassium loss. In addition to the effects on ions, FRU increases blood flow through the renal medulla. This tends to reduce efficiency of the counter-current multiplier system in the loop, since this depends on a hypertonic medulla, resulting in less reabsorption of water from the collecting tubules (32,42,44). In addition, FRU is also thought to cause systemic venous dilatation and hence a reduction in cardiac pre-load. This property is utilized in the treatment of acute pulmonary oedema, where i.v. FRU will reduce pulmonary venous pressure and vascular congestion within a few minutes, well before it has an appreciable diuretic effect (44).

FRU is used to treat a number of conditions including acute pulmonary oedema, acute & chronic renal failure, hypertension, and peripheral oedema associated with cardiac failure, hepatic disease, nephrotic syndrome, and drug use (eg carbenoxolone). FRU is prescribed at a daily dose of

20 to 160mg as a diuretic. In chronic renal insufficiency doses may vary from 250 to 2000mg a day (45,46).

Interactions and Toxicity

FRU diminishes the excretion of lithium whose dose should be halved. Hypokalaemia potentiates the effects of cardiac glycosides and diminishes the effects of anti-arrhythmic drugs such as procainamide & quinidine. The nephrotoxic & ototoxic effects of aminoglycoside antibiotics are potentiated by FRU. Drugs which promote sodium reabsorption eg. oestrogen, can antagonize the effect of FRU. Drugs which promote potassium excretion, eg. corticosteroids, may act additively with FRU to produce serious hypokalaemia. Non-steroidal anti-inflammatory drugs, in particular indomethacin, can interact with FRU, probably via their action on renal prostaglandins, inhibiting the diuretic effect of FRU. FRU in combination with a potassium-sparing diuretic such as amiloride, can cause hyperkalaemia when given concomitantly with an ACE-inhibitor (32,44,47).
The most common side-effect associated with FRU therapy is fluid & electrolyte imbalance and hypotension. Other side-effects are relatively uncommon and include allergy, nausea, diarrhoea, blurred vision, skin rashes, tinnitus & deafness. FRU may provoke hyperuricaemia, and less frequently, hyperglycaemia. FRU therapy in the elderly is particularly likely to provoke hypokalaemia, and malnutrition increases this risk. In elderly women incontinence may be exacerbated by loop diuretics, and elderly men with benign hypertrophy of the prostate may suffer from acute retention of urine following a large volume diuresis. Interference with social activities can also occur (40,47).

FRUSEMIDE DISPOSITION

Absorption and Distribution

FRU is incompletely but fairly rapidly absorbed from the gastrointestinal tract, with a bioavailability of around 61% (48). Following absorption, FRU is extensively bound to the plasma protein albumin, with the usual percentual binding of 97.7% decreasing in renal impairment, advanced illness and old age (49,50,51,52). Peak plasma concentrations occur about 60 minutes after oral ingestion, and although the extent of FRU absorption may be reduced in severe cardiac failure (53,54), age does not appear to influence FRU absorption (55). Effects of an oral dose are seen within an hour of administration, and last for 4-6h. When given i.v. FRU takes effect after 5 minutes, with a duration of action of around 2h (47).

Metabolism and Excretion

FRU has a biphasic half-life, the terminal phase representing elimination, which has a half-life of about 90 minutes (53,56,57,58). Elimination is prolonged in renal impairment, acute pulmonary oedema & congestive heart failure and also in old age (57,58,59,60,61,62). FRU is mainly excreted unchanged in the urine, with active tubular secretion, via the nonspecific organic acid pathway excreting bound FRU, and glomerular filtration eliminating free drug (48). Metabolism to FRU-glucuronide appears to occur, and CSA (4-chloro-5-sulphamoyl-anthranilic-acid) may be another metabolite although this is controversial (58,60 62). FRU is also excreted into the bile (53,61). Non-renal excretion is thought to become more important when renal function is compromised (59,60).

1.4.2 PARACETAMOL

Acetanilide was first introduced into medicine in 1886 as an antipyretic but was found to increase methaemoglobin (32). The search for safer alternatives led to the development of phenacetin & paracetamol, derivatives of acetanilide. Phenacetin was initially very popular until it was found to possess nephrotoxic properties - this led to a subsequent decline in usage (63). From this point on the use of PAR increased and it is now widely used for many minor complaints.

Mode of Action & Therapeutic Uses

Paracetamol (acetaminophen, PAR) is a moderately water & lipid soluble weak organic acid with a pKa of 9.5 which is largely unionised over the physiological range of pH (64). It has antipyretic & analgesic actions similar to aspirin but only a weak anti-inflammatory action (65). Its mode of action may be similar to that of aspirin and dependent on inhibition of prostaglandin synthesis (66). PAR is used to treat a variety of conditions including mild pain, headache dysmenorrhoea & pyrexia. It is invariably taken orally, in an adult dose of 500-1000mg every 6-8h (max 4g/24h). It is often used in combination with other analgesics eg codeine.

Interactions and Toxicity

PAR interacts with phenytoin & phenobarbitone which increase its metabolism, probably through enzyme induction (67,68). PAR metabolism is also increased by desipramine (69) and reduced by chloramphenicol (70).

Adverse affects are rarely seen with therapeutic doses - skin rashes & haematological reactions have been noted (71). Liver damage resulting in jaundice, and kidney

damage may follow chronic use of PAR (66,72). In overdose however, paracetamol can be extremely toxic with only 7g causing acute centrilobar hepatic necrosis, and 15g causing death (47). The mechanism of hepatic toxicity involves a highly reactive metabolite which is usually inactivated by conjugation with hepatic glutathione. Hepatic glutathione is rapidly used up by toxic doses of PAR, and when stores are reduced to less than 30% of normal, excess metabolite is free to combine with cell constituents to cause damage (73). There are no clinical manifestations of poisoning and maximum abnormalities of liver function tests can be delayed for 3 days; the plasma PAR concentration is therefore often used as a guide to prognosis. Exogenous glutathione does not enter cells readily but precursors such as methionine and N-acetyl cysteine can penetrate to combine with the intermediate and avert hepatic toxicity if administered sufficiently early (32).

PARACETAMOL DISPOSITION

Absorption and Distribution

Paracetamol absorption is negligible from the stomach but rapid from the small intestine; peak plasma concentrations occur 30-120 minutes after ingestion of solid dose formulations (12). Absorption rate is influenced by gastric emptying rate which is increased by posture & drugs such as metoclopramide, and reduced by food and drugs such as morphine & loperamide; extent of absorption is unchanged. Age does not appear to affect the rate or amount of PAR absorbed (12,13,74). PAR undergoes dose-dependent first-pass metabolism with 90% bioavailability for a 1g dose, and distributes throughout most tissues & fluids except CSF & fat, with no protein binding at a therapeutic dose (64).

Metabolism and Elimination

PAR is extensively metabolised with only 2-5% of a therapeutic dose appearing unchanged in urine - the major metabolites are sulphate & glucuronide conjugates. A minor fraction is converted by cytochrome P-450-dependent hepatic mixed function oxidase to a highly reactive intermediate, usually inactivated by further conjugation with glutathione which is metabolised into PAR cysteine & PAR mercapturic acid. In healthy subjects approximately 85-95% of a 1g dose appears in urine within 24h; about 4% as unchanged PAR, 55% as glucuronide, 30% as sulphate, 4% as cysteine & 4% as mercapturic acid conjugates (75). These proportions may change in old age; the percentage of PAR glucuronide may increase and the percentage of PAR cysteine decrease with increasing age (76).

PAR is filtered at the glomerulus with subsequent extensive reabsorption, while PAR conjugate clearances suggest active renal tubular secretion (77). Plasma PAR concentration-time curves are multiexponential with a short half-time for the initial distribution phase (about 3-19 minutes). The elimination phase undergoes first order kinetics from 2-12h post-dose, with a half-life in the range of 1.9-2.5h (78). Total body clearance is about 5ml/kg/min in healthy young volunteers but may be reduced in old age (13,74,79,80,81).

1.4.3 DIGOXIN

Digitalis has been in clinical use for many centuries with the first description of its therapeutic effect in cardiac oedema published by William Withering in 1785. The active components of digitalis are collectively termed "cardiac glycosides", and all share an aglycone ring wherein the pharmacologic activity resides, combined with one to four

sugar molecules that modify the pharmacokinetic properties (42,44). All have characteristic electrophysiological & inotropic effects on the heart (82), but isolation of the individual components has led to production of standardized formulations. By far the most commonly prescribed glycoside used in the UK is digoxin, although digitoxin is occasionally used as an alternative. All subjects in the "Digoxin Study" were prescribed digoxin and so the scope of this thesis is limited to discussion of digoxin alone.

Mode of Action and Therapeutic Uses

Digoxin (DIG) is a polar compound consisting of a steroid nucleus with an OH side group (44), and its two main pharmacological properties utilized in therapeutics are :-

(i) the production of complex electrophysiological changes in cardiac conducting tissue to slow heart rate (negative chronotropic effect).

(ii) the ability to increase force & velocity of myocardial contraction (positive inotropic effect).

These two properties lend themselves to the use of DIG in the treatment of supraventricular tachyarrhythmias and CCF. In atrial tachycardias (in particular atrial fibrillation) DIG is singularly effective in reducing heart rate to improve ventricular filling, and this property remains beneficial until heart rate falls below 60bpm. In cardiac failure the principal therapeutic action is the positive inotropic effect on the myocardium. The desirability of this is controversial, particularly long term, since the benefit of reducing heart size and thus reducing oxygen demand is offset by an increase in work load and associated oxygen demand of the failing heart in the face of

myocardial disease (42,82,83,84,85,86). Thus DIG is no longer the drug of choice for patients with heart failure in sinus rhythm (45,47).

Cardiac glycosides inhibit the ATPase responsible for the sodium pump. Their electrophysiological effects are thought to be due to changes in transmembrane potential brought about directly by that inhibition. The effect on cardiac muscle is probably due to changes in intracellular free calcium secondary to changes in intracellular sodium concentrations brought about by that inhibition (44).

DIG has a narrow therapeutic window, and the daily dosage is therefore variable and dependent on a number of factors. When renal function is normal, the recommended maintenance dose is 0.25-0.5mg or 5mcg/kg daily reduced to 0.125-0.25mg or 2mcg/kg daily in old age & renal insufficiency (44,87).

Interactions and Toxicity

A number of drugs have been shown to affect DIG pharmacokinetics and these are summarized in Table 1.4.1 (82,88); Interactions of clinical importance are given in **bold type.**

Table 1.4.1 Agents affecting DIGOXIN pharmacokinetics

Alteration	Agents
Decreased absorption	charcoal, antacids, dietary fibre, neomycin, cytotoxic agents, kaolin, metoclopramide, sulphasalazine
Increased absorption	antibiotics (inhibits gut flora) anticholinergics (propantheline)
Inhibition of renal tubular secretion	**quinidine, quinine, verapamil** spironolactone, trimethoprim, triamterene
Decreased Vd	**quinidine**
Increased [SDIG] (mechanisms unknown)	**amiodarone**,aspirin, diltiazem, indomethacin, **nifedipine**, flecainide, nicardipine

In addition to these pharmacokinetic interactions, other drugs may give rise to pharmacodynamic interactions. Hypokalaemia is associated with an increased myocardial sensitivity to DIG (89), which may be induced by a variety of drugs eg. corticosteroids, potassium wasting diuretics, acetazolamide and carbenoxolone (45,46). Myocardial responsiveness may also be enhanced by other electrolyte disturbances eg. hyperkalaemia, hypercalcaemia and hypomagnesaemia, also hypoxia & acidosis (88).

Disease states known to affect response to DIG are given in Table 1.4.2 (42,82). Although old age is not especially associated with increased myocardial sensitivity, DIG elimination rate is reduced due to the inevitable decline in renal function (90,91,92). In addition, DIG Vd is reduced in old age and changes in body composition also influence DIG distribution to increase [SDIG] (9,10,93).

Table 1.4.2 Effect of disease state on response to DIG

Disease State	Altered Response
Renal disease	decreased DIG elimination & Vd
CCF	decreased DIG elimination increased Vd in oedematous patient
Hyperthyroidism	reduced myocardial sensitivity increased renal elimination & Vd
Hypothyroidism	enhanced myocardial sensitivity reduced renal elimination & Vd
Gastrointestinal disease	decreased absorption, vomiting & diarrhoea may reduce $[K^+]$
Muscle wasting disease	reduced binding to skeletal muscle
Pulmonary disease	increased myocardial sensitivity during hypoxia & acidosis
Acute MI	increased myocardial sensitivity
Hepatic disease	no significant changes
Diabetes insipidus	no significant changes
obesity	no significant changes

Although knowledge of [SDIG] is generally thought to be useful in the diagnoses of both DIG toxicity & sub-maximal therapy, interpretation depends not only on the absolute level but also on the clinical status of the subject, since presence of factors known to influence myocardial response affect the clinical outcome. This is discussed in chapter 6

Toxicity is dose related, but the threshold at which signs first appear varies greatly between individuals (89,94). Symptoms of DIG toxicity fall into five categories as given in Table 1.4.3 (42,46,85,93). Moreover, the less serious manifestations of toxicity do not serve as a reliable warning of cardiotoxicity which is potentially fatal (93).

Table 1.4.3 Clinical features of digoxin toxicity

System	Signs & Symptoms
Gastrointestinal	anorexia, nausea, vomiting, diarrhoea salivation
Neurological	malaise, fatigue, confusion, facial pain, insomnia, depression, vertigo, hallucinations, dizziness, coloured and hazy vision (green or yellow haloes), transient psychosis, sweating
Cardiological	palpitations, arrhythmias, syncope bradycardia, heart block
Blood	high SDIG level with low potassium
Miscellaneous	gynaecomastia, skin reactions

DIGOXIN DISPOSITION

Absorption and Distribution

Limited absorption takes place from the stomach and the majority is passively absorbed from the proximal part of the small intestine (95,96) with peak plasma concentrations seen between 45 & 105 minutes after ingestion of a solid dose formulation (82,91). Factors which influence the rate

of absorption have little effect on the total amount absorbed, but those which influence the extent of DIG absorption determine [SDIG] at all times (97), as discussed in chapter 6.

Following ingestion, 60-70% of the administered dose is available for systemic circulation in most subjects, although this figure can vary, sometimes due to disease, gut flora or tablet formulation (44,47,82,91,98,99,100,101) DIG is widely distributed and so Vd is large (6l/kg), but may be reduced in old age & renal insufficiency and increased in hyperthyroidism (82,91,93,98,102,103,104,105, 106). The largest proportion of circulating DIG is taken up by skeletal muscle, with the liver, heart, brain & kidneys also binding smaller amounts (107). DIG is 20% bound to plasma proteins but the large Vd makes any interaction at the site of binding clinically unimportant (47,92,98). DIG does not bind to adipose tissue and dosage calculations are more reliable if based on lean body mass rather than actual bodyweight for obese subjects (108,109).

Metabolism and Elimination

Although DIG is usually reported to be excreted unchanged in urine, evidence suggests that metabolism may at times be extensive. A number of metabolites have been detected in urine, particularly after chronic dosing, when detection is facilitated by their long elimination half-life (97,98,110, 111). The degree of metabolism varies between subjects, is independent of renal function, and possibly under pharmaco-genetic control (110,112).

In most subjects however, renal excretion of unchanged DIG by passive glomerular filtration & active tubular secretion is the most important route of elimination (82,92,98).

Approximately one third of the dose absorbed may be excreted by nonrenal routes eg secretion into bile (82). Following i.v. administration, DIG pharmacokinetics may be described by a two- or three-compartment model, although steady state kinetics after multiple dosing may be sufficiently described by a one-compartment model (44,92,93 105,113). Distribution is complete 6h after ingestion, and blood sampling for [SDIG] estimation should be carried out after this time (114). Elimination half-life is variable and related to renal function (91,93,104); estimates of mean $t_{1/2}$ have varied eg. 37h in healthy young subjects, 70h in nontoxic elderly people, 79h in those with renal impairment, and the highest values for DIG $t_{1/2}$ have been reported as 118h in elderly people with symptoms of toxicity, and 189h in a subject with renal insufficiency (91,93,98,102,104). Total body clearance has also been found to decline in old age & renal impairment (91,98,104). Due to the long $t_{1/2}$, attainment of steady-state commonly takes in excess of a week if a fixed daily dose of DIG is administered (44). In emergencies a therapeutic [SDIG] level may be obtained more quickly by administration of a loading dose, eg. 15mcg/kg in divided doses over 12h (44, 45). However, this is often associated with an increased incidence of gastrointestinal side effects and slower digitalisation is preferable in most non urgent cases (47).

1.5 SCOPE OF THE THESIS

To even the most casual observer a substantial heterogeneity in the abilities of old people can be seen to exist, although in the past many studies have considered "the elderly" to form a single homogeneous group. Whilst

age-related changes in renal & hepatic function have been extensively investigated, most studies have used "fit" elderly subjects, often ill defined, and the results extrapolated to encompass the entire elderly population. However, recent research has suggested that hepatic & renal function, and consequently drug clearance, may differ between those who are fit & active and those who are frail & immobile (28).

Many elderly people living in the community have an appearance of "fitness" whilst having a well controlled chronic disease which requires medication but causes little interference with activities of daily living. Conversely, those elderly people frequently found in nursing homes or long-stay hospital wards have an appearance of "frailty", seeming less well than their active counterparts despite being of similar age and having comparable diagnoses. In addition, those in care tend to be prescribed more drugs and be more severely restricted in their mobility and ability to carry out activities of daily living. Attempts have been made to define particular sub-groups of old people but none appear to encompass the majority. Therefore, the aim of this thesis was primarily to identify and define distinct groups of elderly people who together form the majority of the aged in the community and in care; the two resulting groups were termed "fit" and "frail".

Elderly people consume a disproportionate quantity of drugs and both groups studied were frequent users of prescribed medication. It is often desirable to know a patients renal function before renally excreted drugs are prescribed but CCr is often difficult to accurately measure in elderly patients, particularly when confused or incontinent.

CCr was measured in both fit & frail groups and compared to determine whether renal function differed significantly between the groups when matched for sex, age & weight. The estimation of CCr from a single SCr and use of a mathematical equation has been offered as an alternative, with results readily obtained from minimal effort. Since renally excreted drugs are commonly prescribed to both fit & frail elderly people, the accuracy with which CCr is estimated in these groups was examined, using a variety of equations and timed urine collections of less than 24 hours duration.

Following this, the efficiency of renal clearance of frusemide and digoxin was examined and compared between groups. Unfortunately, hepatic function cannot be measured as readily as renal function and so comparable studies could not be conducted to determine whether differences in hepatic function existed between the two groups of elderly people. Therefore, paracetamol was chosen as a model drug and the efficiency of its hepatic clearance measured and compared between the fit & frail groups.

To summarise, the aim of this work was to identify, define and compare groups of fit & frail elderly people in terms of renal function, and hepatic and renal drug clearance. Since frail elderly patients seem to exhibit an excess of adverse drug reactions when compared to their fit counterparts, increased understanding of the differences in efficiency of drug clearance between fit & frail elderly people may reduce the incidence of drug-related morbidity & mortality in those at greatest risk.

CHAPTER TWO

MATERIALS and METHODS

2.1 COMPOUNDS

Drugs

i) Frusemide tablets BP 40mg (non-proprietary brands, supplied by patients own Pharmacy on prescription)

ii) Frumil tablets (frusemide 40mg, amiloride HCl 5mg, Rorer Pharmaceuticals, Eastbourne, Sussex. Various lots supplied by patients Pharmacist on prescription)

iii) Lanoxin tablets (62.5, 125 or 250micrograms Digoxin BP Wellcome Medical Division, Crewe, Cheshire. Various lots supplied by patients Pharmacist on prescription)

iv) Paracetamol tablets BP 500mg (Sterwin Medicines, Guildford. Lot 1EF539, supplied by Pharmacy Dept, St. Martin's Hospital, Bath.)

Analytes

i) Creatinine 10.0mmol/l in 0.1M HCl, $C_4H_2N_3O$ mw=113 (BDH Chemicals Ltd., Poole. Lot 777812OH)

ii) Creatinine 0.088, 0.265 & 0.883mmol/l in 0.02M HCl (Sigma Chemical Company. Lot 128F-6149)

iii) Frusemide BP (furosemide) $C_{12}H_{11}ClN_2O_5S$ mw=330.8 (Sigma Chemical Company. Lot 26F-0636)

iv) Paracetamol BP (4-acetominophenol, acetaminophen) $CH_3CONHC_6H_4OH$ mw=151 (Pharmacy, University of Bath)

Solvents

i) acetonitrile (methyl cyanide) HPLC grade CH_3CN mw=41 (Fisons, Loughborough)

ii) isopropanol HPLC grade $(CH_3)_2CHOH$ mw=60 (Fisons)

iii) methanol HPLC grade CH_3OH mw=32 (Fisons)

Kits

i) Coat-A-Count Digoxin containing digoxin antibody-coated tubes, [^{125}I] digoxin & calibrators 0-8 ng/ml (Diagnostic Products Corp., USA. Lot TKDI1 365 & 387).

Other Chemicals

i) acetic acid AR grade CH_3COOH mw=60.1 (Fisons)

ii) beta-glucuronidase enzyme from Helix pomatia. activity: 440000 beta-glucuronidase, 15000 sulphatase units/g solid (Sigma. Lot 88F-7325)

iii) orthophosphoric acid 88% AR H_3PO_4 mw=98 (Fisons)

iv) potassium dihydrogen orthophosphate KH_2PO_4 mw=136 (Fisons)

v) sodium acetate HPLC grade CH_3COONa mw=136.1 (Fisons)

vi) sodium hydroxide AR grade NaOH mw=40 (BDH)

2.2 HUMAN VOLUNTEERS

Studies were carried out in accordance with the Declaration of Helsinki (Venice revision 1983) and studies received approval from the Bath District Research Ethical Committee. Before participation in a study, approval was given by the subjects Consultant Geriatrician or General Practitioner. Volunteers gave informed consent (verbal witnessed or written) and were free to withdraw from the study at any time without explanation. Subjects of both sexes aged between 64 & 100 years were recruited from

a) Wards 1, 2, 3, 4, 21 & 22 of St. Martins Hospital, Bath.

b) Patients registered with General Practitioners at St. Mark's Road Surgery, Widcombe, Bath.

c) Subjects on the volunteer panel of the Research Institute for the Care of the Elderly, St. Martins Hospital, Bath.

Exclusion criteria were as below :-

a) inability to give informed consent

b) incontinence

c) an acute episode of illness

d) co-medication with drugs interacting with the study drug

Records were made of the following :-

a) date of birth and age to nearest year

b) height & weight (surface area calculated from this)

c) all drugs currently being taken (prescribed or over-the-counter), the dose & frequency.

d) diagnoses and blood results if available

e) approximate amount of meat consumed during the study

The mobility of each subject was assessed during the study using a mobility rating scale devised for the studies (appendix A2). From their independence, mobility & ability to carry out activities of daily living, each subject was also categorized as fit or frail according to the definitions used during these studies (appendix A1).

2.2.1 Administration of drugs

Drugs were administered orally under supervision, taken with a glass of water, in the upright position. Drugs were given in the morning after a light breakfast eaten at least one hour previously, unless otherwise stated. Urine was voided immediately prior to dosing when the time was noted.

2.2.2 Treatment & storage of biological samples

Samples were processed as soon as possible after collection Urine was collected into a plastic jug or bottle for continent subjects, or into a catheter bag for catheterised patients, and saved in sealed plastic bottles labelled with

the subjects name and time of voiding. Urine volume was measured and, when required, grouped into aliquots each spanning about 6-8h, before freezing. Blood was taken using plain Vacutainer tubes, labelled, and left to clot for one hour before being spun at 2000rpm for 15m. Serum was then collected & frozen. Frusemide-containing samples were wrapped in silver foil and processed in subdued light. Samples were stored at -20^{o}C prior to analysis; serum in 2x5ml or 2x1.5ml plain glass tubes and urine in 2x20ml or 2x1.5ml aliquots. Samples were defrosted once just before use, and used within their stability period; 6 months for creatinine, 2 months for FRU & DIG, 6 weeks for PAR.

2.3 INSTRUMENTATION

During periods of analytical work balances were checked monthly, and pipettes weekly, for accuracy and constancy.

2.3.1 Radioimmunoassay

Radioactivity was measured using a LKB Wallac 1275 Gamma Counter (Pharmacia Ltd, Milton Keynes). The counter was set to the appropriate isotope (^{125}I) and counts measured over 60s. Equipment was operated at ambient temperature.

2.3.2 Analysis of Digoxin

DIG was assayed using a commercially available RIA kit. Serum DIG was analysed as per the data sheet (appendix B1). Although the diagnostic kit was intended for use with serum the method was validated using urine diluted with water, and thus the same method was employed to measure urinary DIG. Calibrators supplied were used to construct calibration curves. Urine was diluted 1 in 10 or 1 in 20 to ensure that readings fell within the calibration range.

Quantitation of Digoxin in serum & urine

The mean net count for each calibration tube and patient sample were calculated as below :-

net counts = mean count - mean NSB count

The binding for each concentration was then found as a percent of maximum binding (MB), with the nonspecific binding (NSB) corrected counts of the A tubes taken as 100%

percent bound = (net counts / net MB counts) x 100

Using logit-log paper percent bound was plotted against concentration for each of the calibrators to form a line. Digoxin concentrations for the unknowns were then estimated from the line by interpolation.

2.3.3 High Performance Liquid Chromatography

HPLC is a technique used to separate components of a chemical mixture. Components are initially dissolved in liquid solvent and forced to flow through a chromatographic column at a stable pressure of normally between 1000-3000 psi. Following application onto the column, the mixture is resolved into its components which are separately eluted & selectively detected using a uv monitor. This technique is therefore amenable to the separation of compounds which occur naturally as a mixture eg. biological fluids. The specificity of the system is such that components can be quantified to a high precision.

Mobile phase was pumped at a constant flow rate by a CM4000 pump (Laboratory Data Control, (LDC), Stone, Staffs), which provides a pulseless flow using dual-reciprocating pistons. Samples were either injected onto the column using a Promis II autosampler (LDC) or via a rheodyne valve (model 7125), fitted with a 20ul or 100ul loop. Detection was by a

SM4000 variable uv wavelength detector (LDC) and signals were recorded & plotted by a CI10 (LDC) printer/integrater. Mobile phase was made up using high purity salts (HPLC grade) and double distilled deionised (DDDI) water. After adjustment to the required pH, mobile phase was filtered through a 0.45um membrane filter (Millipore), and before use degassed by purging with helium for 10m. The columns (15cm or 25cm x 4.5mm internal diameter) were constructed from stainless steel tubing, and packed with reversed phase micro-particle (5um) silica (Hypersil ODS, Shandon Ltd., Cheshire). Equipment was operated at ambient temperature and all samples were run in duplicate.

No internal standards were employed as sample preparation in all cases was by simple dilution. Calibration curves were constructed for each drug in urine & serum appropriately diluted, and the correlation coefficient r, gradient m, & intercept c, were calculated by linear least-squares regression. For linearity to be assumed over the chosen range r was required to be near to unity ($r > 0.980$)

2.3.4 Analysis of creatinine

Creatinine was assayed in urine (U) & serum (S) by HPLC using a modification of the method by Ginman & Colliss, 1985 (115).

Chromatographic conditions

Wavelength	233nm
Range	0.1
Flow rate	1ml/m
Chart speed	5mm/m
Column	RP Hypersil 5um ODS 25cm x 4.5mm i.d. (LDC)

Mobile Phase

urine 12% CH_3CN : 88% 0.05M CH_3COONa (6.80g/l) to pH 6.5

serum 0.2% CH_3CN : 99.8% 0.05M CH_3COONa to pH 7.5

pH adjustment by glacial acetic acid

Using the above conditions the retention time was 2.4 & 3.2 minutes for creatinine in human urine & serum respectively, and typical chromatograms are given in Fig. 2.3.1

Stock Solutions	**Working Solutions**
0.088 mmol/l creatinine	same
0.265 mmol/l creatinine	same
0.883 mmol/l creatinine	same

Sample Treatment

(i) Urine Analysis

100ul or 50ul U + 900ul (U/10) or 950ul (U/20) DDDI

↓ mix, 100ul U/10 or U/20 + 200ul CH_3CN + 800ul DDDI

↓ mix, centrifuge for 10m at 13000rpm

20ul taken for HPLC analysis

(ii) Serum Analysis

100ul serum + 200ul CH_3CN + 500ul DDDI water

↓ 100ul water or 100ul 0.088 mmol/l Cr

↓ mix, centrifuge for 20m at 13000rpm

20ul taken for HPLC analysis

Calculation of Serum Creatinine Concentration

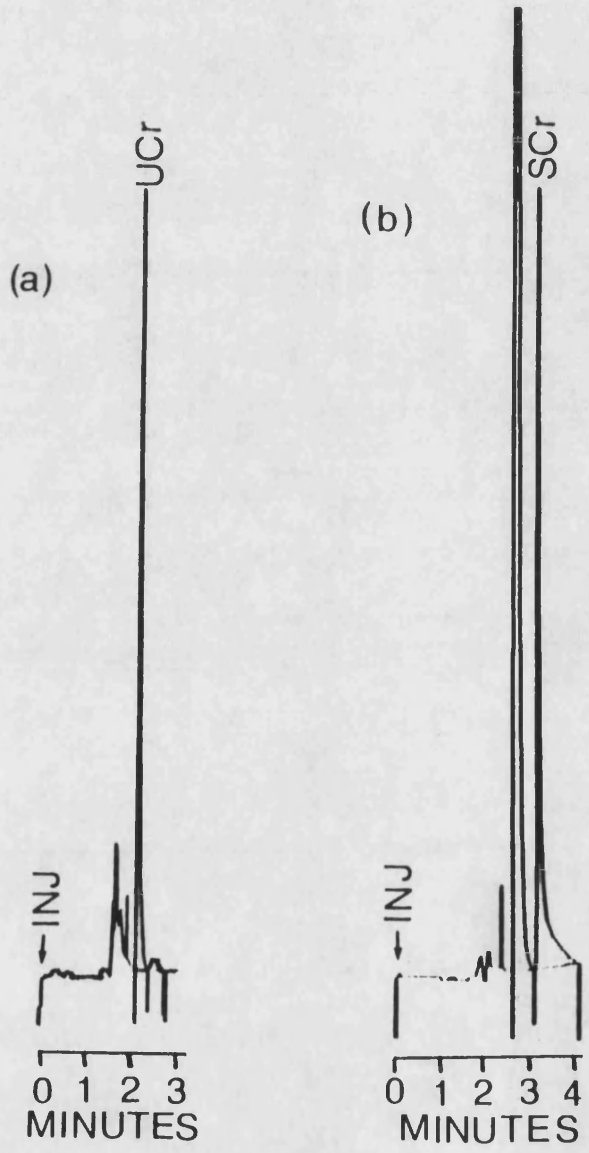
Linearity over the expected range of creatinine in serum was verified. Due to the wide interindividual variation in SCr, [SCr] was determined by "spikeing" samples with creatinine, and comparing the mean peak height of duplicate samples before and after "spikeing". The concentration of creatinine in each sample was determined by the equation overleaf :-

Fig. 2.3.1

Typical chromatograms of CREATININE in

(a) human urine and

(b) human serum



$m = y_2 - y_1$

where y_1 = mean unspiked peak height

y_2 = mean spiked peak height

m = gradient of line (linear from standard curve)

then $x = y_1/m$ where x is unknown SCr in unspiked sample

Standard Curve

The calibration curve was constructed from 0-40ug/ml and was linear over this range for peak height & area (r=0.998)

The coefficient of variation was i) 0.8% at 1ug/ml (n=10)

ii) 0.7% at 40ug/ml (n=10)

2.3.5 Analysis of frusemide

Frusemide (FRU) was assayed in urine & serum by HPLC using a modification of the method by Karreman et al 1982 (116).

Chromatographic conditions

Wavelength	230nm
Range	0.01
Flow rate	1ml/m
Chart speed	5mm/m
Column	RP Hypersil 5um ODS 15cmx4.5mm i.d. (Jones)

Mobile Phase

53% methanol : 47% 0.02M KH_2PO_4 (2.72g/l) to pH 3.0

pH adjustment by orthophosphoric acid

Analyses were carried out in subdued light. Using the above conditions the retention time was 3.48m for FRU in urine & serum; typical chromatograms are given in Fig 2.3.2

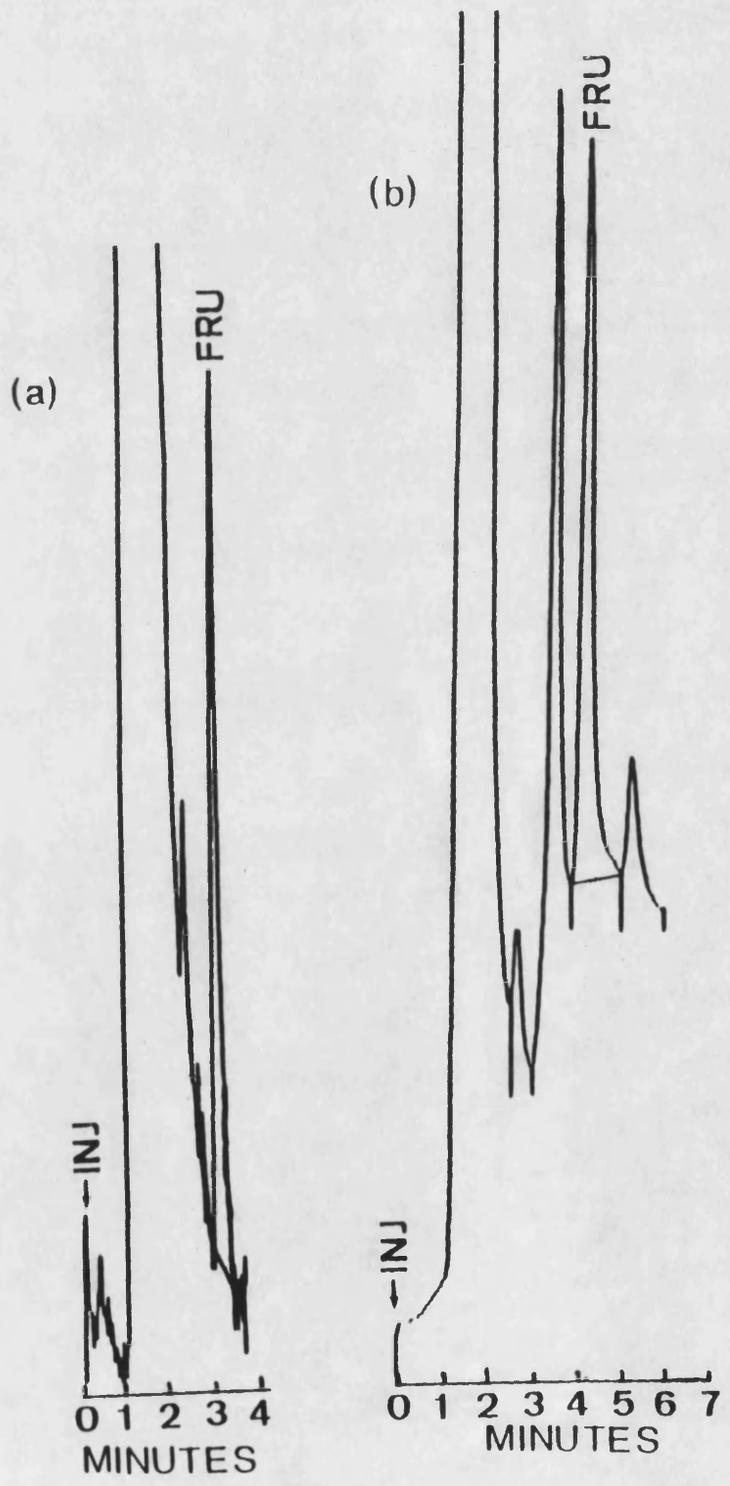
Stock Solution	**Working Solutions**
(a) 1mg/ml FRU in methanol	(a) diluted 1/10 & 1/100 with H_2O

Fig. 2.3.2

Typical chromatograms of FRUSEMIDE in

(a) human urine and

(b) human serum



Sample Treatment

The following were added to an eppendorf tube :-

(i) **Urine Analysis**

50ul urine + 950ul DDDI water

↓ mix, centrifuge for 10m at 13000rpm

20ul taken for HPLC analysis

(ii) **Serum Analysis**

500ul serum + 500ul CH_3CN

↓ mix, centrifuge for 20m at 13000rpm

20ul taken for HPLC analysis

Standard Curve

A calibration curve was constructed from 0 - 2.0 ug/ml and was linear over this range for peak height & area (r=0.990) The coefficient of variation was i) 3.1% at 0.1ug/ml (n=10)
ii) 2.3% at 2.0ug/ml (n=10)

2.3.6 Analysis of paracetamol

Paracetamol was assayed in human urine (U) & serum (S) by HPLC using a modification of the method by Adriaenssens 1978 (117).

Chromatographic conditions

Wavelength	254nm
Range	0.1(U) 0.02(S)
Flow rate	1ml/m
Chart speed	5mm/m
Column	RP Hypersil 5 ODS 15cm x 4.5mm i.d. (Jones)

Mobile Phase

urine 5% isopropanol : 95% 0.08M KH_2PO_4 (10.9g/l) to pH 3.0

serum 3% isopropanol : 97% 0.08M KH_2PO_4 to pH 3.0

pH adjustment by orthophosphoric acid

Using the above conditions the retention time was 4.68 & 3.28 minutes respectively for PAR in human urine & serum, and typical chromatograms are given in Fig. 2.3.3.

Stock Solution

(a) 1mg/ml PAR in methanol

Working Solutions

(a) diluted 1/10 & 1/100 with H_2O

Sample Treatment

The following were added to an eppendorf tube :-

i) Urine Analysis

100ul urine + 900ul DDDI water (U/10)

↓ Mix, 100ul U/10 + 200ul CH_3CN + 900ul DDDI water

↓ Mix, centrifuge for 10m at 13000rpm

100ul taken for HPLC analysis

ii) Urine Analysis of Paracetamol Glucuronide & Sulphate

Paracetamol glucuronide & sulphate conjugates were assayed as PAR following enzymatic hydrolysis (at 37°C for 24h) by sulphatase and beta-glucuronidase.

iii) Serum Analysis

100ul serum + 200ul CH_3CN + 900ul DDDI water

↓ Mix, centrifuge for 20m at 13000rpm

20ul taken for HPLC analysis

Standard Curve

The calibration curve was constructed from 0 - 15 ug/ml and was linear over the range for peak height & area (r=0.990)

The coefficient of variation was i) 3.2% at 0.2ug/ml (n=10)

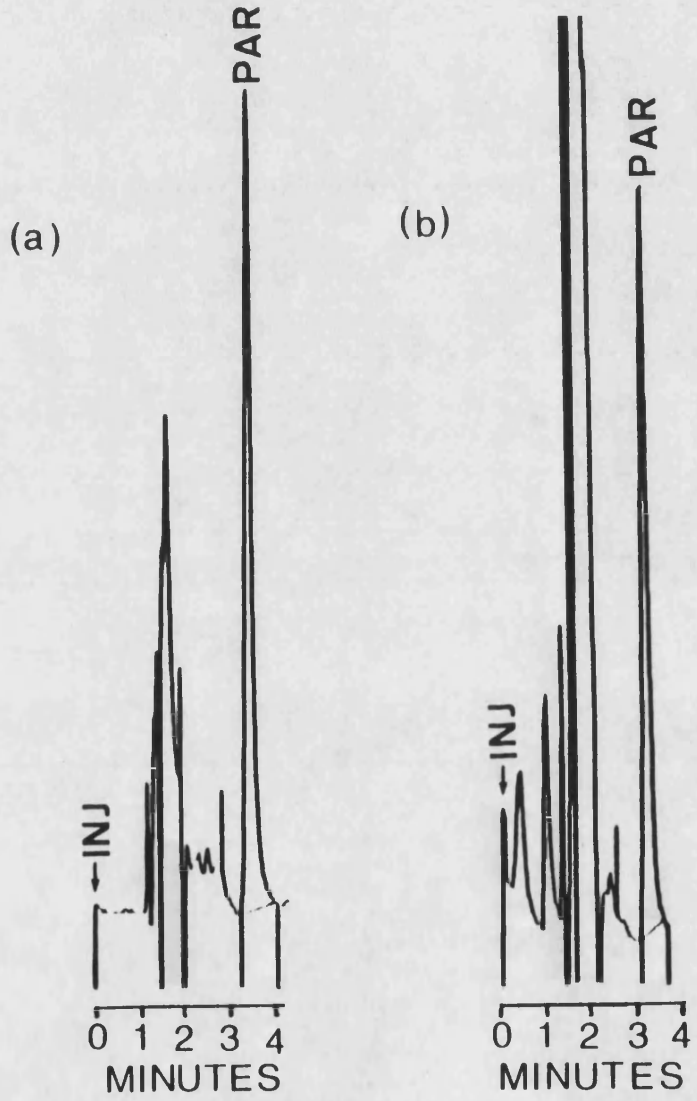
ii) 0.7% at 10ug/ml (n=10)

Fig. 2.3.3

Typical chromatograms of PARACETAMOL in

(a) human urine and

(b) human serum



2.4 STATISTICAL EVALUATION & TREATMENT OF RESULTS

Statistics

Statistical analyses were performed using the computer package "Minitab" version Release 7.

Results were expressed as means & standard deviations (s.d.). Upper & lower quartiles (Q3 & Q1) and medians were also quoted in particular instances.

Statistical difference between two means was determined using the Mann-Whitney U test with statistical significance occurring at a probability of $p<0.05$ (5%).

Correlations between parameters were determined using Pearsons correlation coefficient for normally distributed samples, or when $n > 50$, or Spearmans coefficient of rank correlation for non-normally distributed samples and when $n < 50$. Due to the large number of correlations performed within each data set (eg. 15 columns x 14 rows) it was highly likely that Type II errors (ie. null hypothesis falsely accepted) would occur by chance. To reduce this probability statistical significance was taken to occur at $p<0.01$ (1%) for Pearsons & Spearmans correlations.

Calculations

Elimination half-life, $t_{1/2}$, was calculated from the plot of log [drug] v time, where kel is the elimination rate constant from $m = \frac{-kel}{2.303}$ then $t1/2 = \frac{0.693}{kel}$

Serum clearance, $\frac{Cls = \text{dose x fraction absorbed}}{AUC}$

- AUC is the area under the [serum] v time curve

Renal clearance, $Clr = \frac{\text{dose recovered in urine}}{[drug]}$

- [drug] is the concentration of drug in serum at the mid-time point for the urine collection.

Creatinine clearance, $CCr = \frac{UCr \times v \times 100}{SCr \times 1440}$

- UCr is the urinary creatinine concentration (mg/100ml)
- v is the urine flow rate (ml/24 hours)
- SCr is the serum creatinine concentration (mg/ml)

Body surface area, $SA = A_o.H^{a1}.W^{a2}$

- A_o is 0.0235
- H is the height in cm, a1 is 0.42246
- W is the weight in kg, a2 is 0.51456

CHAPTER THREE

CREATININE CLEARANCE IN ELDERLY PEOPLE

3.1 INTRODUCTION

For drugs whose elimination is accomplished entirely or partly by renal excretion, total clearance will decline in proportion to the inevitable reduction in GFR occurring in old age. It is often desirable to know a patients' GFR before such drugs are prescribed to prevent accumulation, particularly when the therapeutic window is narrow. In clinical practice renal function is often estimated by measurement of creatinine clearance (CCr), involving the assay of creatinine in serum & a 24h urine sample. Alternatively, specially derived formulae may be utilized to predict CCr from serum creatinine alone, with the advantage of speed & convenience. Few studies have addressed the question of whether these "short-cut" methods are valid in the elderly, when inaccuracy of prediction may lead to inappropriate drug therapy. In this study CCr was measured in an heterogeneous group of old people and compared with the predicted CCr obtained from SCr and use of equations. In addition CCr was calculated from urine collections of about 8h, carried out at various times of day, and their accuracy to predict 24h CCr compared. The influence of mobility & frailty on CCr and its prediction were also investigated.

3.2 PROCEDURE

Very fit, fit & frail people, as defined in appendix A1, aged 60 years and over were invited to take part in the study. Exclusion criteria are given in 2.2. Subjects were

recruited specifically for the CCr study from patients in St. Martins hospital, from General Practice, and from the Research Institute volunteer panel. In addition, subjects taking part in the Frusemide, Paracetamol & Digoxin studies (chapters 4, 5 & 6) were included in the present study since 24h urine collections were also necessary, providing the opportunity to maximize the use of collected material. Each subject was provided in advance with an information sheet (appendix C1, standard or enlarged type) and given the opportunity to question Dr L Parker (GP trainee) or myself about the study prior to its commencement. On the morning of the study subjects emptied their bladder, noted the time, and from then on collected all urine for 24h, either in a single vessel or in a separate container per specimen, labelled with the time of voiding. When the latter method was employed the separate samples were later combined into 3 aliquots, each of approximately 8h, the final aliquot spanning the time spent in bed overnight. On completion of the urine collection, blood was taken in the morning after an overnight fast or meat-free breakfast. Forms given in appendix C2 (urine in aliquots) & C3 (single 24h urine collection) were completed during the study. Samples were assayed for creatinine as per 2.3.4.

During the study subjects were questioned about their daily activities and mobility, and were categorized as very fit, fit or frail and had their mobility scored according to the mobility rating devised for the study (appendix A1 & A2). Current medication was noted and subjects were weighed and had their height measured where possible. Drugs taken & demographic details are given in appendices C4,D3,E3,F3.

Correlations were determined using Pearsons correlation with significance levels being taken at $p<0.01$ for reasons outlined in 2.4. Groups (males v females and fit v frail) were compared using the Mann-Whitney test with significance levels being taken at $p<0.05$.

3.3 RESULTS OF CREATININE CLEARANCE STUDIES

245 subjects (146F) successfully participated in the study to compare measured with predicted CCr, 194 (116F) of whom collected urine in aliquots enabling CCr from 24h & 8h urine collections to be compared. 115 subjects (71F) took part in the CCr study only, 34 (21F) also took part in the frusemide study, 49 (30F) in the paracetamol study & 47 (24F) in the digoxin study. For the CCr study 35 (21F) subjects were very fit, 113 (70F) fit & 97 (55F) frail. The 11 equations & 1 nomogram used to predict CCr from SCr are given in Fig. 3.1.

Comparison of Males and Females

Data from males & females were compared with results given in appendix C6 & Figs 3.2 to 3.4. No significant difference was seen between sexes for age, M.Sc, urine vol or CCr/SA but the groups differed in every other respect. The males weighed significantly more, had a greater SA & excreted more UCr. Minimum, maximum & mean SCr values were also greater in the male group, likewise CCr although CCr/SA was similar between groups. These results suggest that males produce and excrete more creatinine, probably due to their increased weight & muscle mass, giving rise to disparate values for UCr, SCr & CCr. Because of these differences data from males & females were analysed separately.

Females

Individual results for all 24h urine collections are given in appendices C5, D5, E5 & F5. Results from the entire female group are summarized in appendix C7 and correlations between parameters are given in appendix C8.

Age ranged from 60-100y (77±8; mean±s.d.) and significantly correlated with M.Sc, weight, urine vol, UCr & CCr.

Mobility score ranged from 1-5 and significantly correlated with urine volume, UCr, SCr & CCr.

UCr ranged from 240-2441mg (823±313) and correlated with age, M.Sc, weight, urine volume & CCr. SCr varied from 0.34-4.32mg/100ml (1.39±0.69), correlating with M.Sc & CCr.

CCr ranged from 7-141ml/min (48.7±24.1), correlating with age, M.Sc, urine volume, UCr & SCr. Similar correlations were seen for CCr/SA.

116 female urine collections were stored as aliquots enabling CCr from collections of less than 24h to be calculated. The 24h collection was divided into a morning collection (CCram), afternoon/evening collection (CCrpm), and overnight collection (CCrn), each varying in length according to the pattern of urine excretion exhibited by the subject. In all cases CCrn was calculated from urine produced during the time spent in bed overnight. Collection periods ranged from 2.42-11.00h for CCram, 2.08-14.75h for CCrpm, and 4.25-18.00h for CCrn for females & males.

Differences between 24h CCr & CCr from reduced collections was determined for each subject ie CCr - CCram = dCCram; a negative value would result if predicted CCr>measured CCr.

CCr from reduced collection times were also expressed as %24h CCr ie. CCr/CCram x 100 = %CCram; a value less than 100 would result when predicted CCr > measured CCr.

CCram, CCrpm & CCrn are given in appendix C7 with the correlation coefficient between each reduced collection & 24h CCr and the equation of the resulting line. Plots of measured v predicted CCr for each of the three reduced urine collections are given in Figs. 3.3.1 to 3.3.3.

For the 116 females with CCr calculated from reduced urine collection times, 24h CCr varied from 7-141ml/min (49±26). CCram varied between 1-160ml/min (51±30) dCCram varied from ⁻57-36ml/min (⁻2.0±13.3) and %CCram from 5-263% (103±32). The correlation coefficient between CCr & CCram was 0.901; the line had a gradient of 1.05 and y intercept of ⁻0.45. CCrpm varied between 2-156ml/min (52±32) dCCrpm ranged from ⁻80-39ml/min (⁻3.3±15.3) while %CCrpm varied from 20-332% (107±40). The correlation coefficient between CCr & CCrpm was 0.877, the gradient was 1.06 and the intercept 0.37. CCrn varied from 6-153ml/min (46±26), dCCrn ranged from ⁻41-42 ml/min (2.4±10.3) and %CCrn from 27-241% (96±25). The correlation coefficient between CCr & CCrn was 0.922, the gradient was 0.93 and the intercept 1.21.

CCr, CCr/SA or CCr/70kg were calculated using 11 equations & 1 nomogram numbered E1 to E12 and where applicable female modifications are expressed as ExF. Formulae & authors are given in Fig. 3.1. Results are given in appendix C7 with the correlation coefficient and equation of the regression line between measured & predicted CCr for each formulae. Measured v predicted CCr were plotted and are given in Figs. 3.3.4 to 3.3.23.

The "ideal" formula, when plotted against measured CCr, would have a correlation coefficient of 1, a gradient of 1 and an intercept of 0. No formula exhibited these

characteristics and so the 5 "best fit" formulae were selected for further investigation. Female modifications in all except one case did not increase the accuracy of prediction of CCr, and so 4 formulae chosen were common with those suggested for males. Equations selected were E1, E4, E9, E10 & E11F. Plots of measured CCr v %CCr for the reduced urine collections and 5 best fit equations are given in Figs. 3.3.24 to 3.3.31.

Males

Individual results for all 24h urine collections are given in appendices C5, D5, E5 & F5 and results from the male group are summarized in appendix C9. Correlations between parameters are given in appendix C10.

Age ranged from 60-97y (76±8) and significantly correlated with M.Sc, SA, urine vol, UCr & CCr.

Mobility score ranged from 1-5, correlating with age, weight, SA, urine volume, UCr, SCr & CCr.

UCr ranged from 302-2624mg (1192±433), correlating with age, M.Sc, weight, SA, urine volume & CCr. SCr varied from 0.55-6.71mg/100ml (1.67±1.01), correlating with CCr & M.Sc. CCr ranged from 10-193ml/min (61±30) & CCr/SA from 10-164 ml/min/1.73m^2 (56±26). Both correlated with age, M.Sc, weight, SA, urine volume, UCr & SCr.

78 male urine collections were stored as aliquots enabling CCr from collections of less than 24h to be calculated, and results are shown in appendix C9.

For these subjects 24h CCr ranged from 10-193ml/min (60±31) CCram varied from 6-195ml/min (68±41) with dCCram ranging from -87-17ml/min (-8±22) & %CCram from 30-350% (112±49). The correlation coefficient between CCr & CCram was 0.846,

the gradient was 1.11 and a y intercept 1.6.
CCrpm varied between 9-209ml/min (63±37) dCCrpm from -75-29 ml/min (-4±14), %CCrpm from 37-274% (105±30). The correlation coefficient was 0.925, the gradient 1.09 & the intercept -1.5.
CCrn ranged from 3-186ml/min (54±30), dCCrn from -43-50 ml/min (6±14), %CCrn from 7-149% (92±22). The correlation coefficient was 0.905, the gradient 0.87 and intercept 1.8. Plots of measured v predicted CCr for each of the reduced urine collections are given in Figs. 3.4.1 to 3.4.3.

CCr, CCr/SA or CCr/70kg were calculated from SCr using 11 equations & 1 nomogram as in Fig. 3.1. Results are given in appendix C9 with the correlation coefficient & equation of the line between measured & predicted CCr for each formulae. Measured v predicted CCr were plotted and given in Figs. 3.4.4 to 3.4.15.
No formula was "ideal" and so the 5 best fit formula were selected - these were E1, E4, E9, E10 & E11. Plots of measured CCr v %CCr for each reduced urine collection & the 5 best fit equations are given in Figs. 3.4.16 to 3.4.23.

3.3.1 Influence of Diuretics on CCr Prediction

Females

Females were divided into groups according to whether or not they took diuretics and results are shown in appendix C11. The 60 females who took diuretics during the study were older and less mobile than the 86 who did not. CCr tended to be lower in those taking diuretics but this was not significant. CCram, CCrpm & CCrn were reduced for those taking diuretics, but this was only significant for CCrpm ($p<0.02$). dCCrn tended to be greater in those who

did not take diuretics ($p<0.05$), but dCCram & dCCrpm were unchanged. The accuracy of CCr prediction using 5 selected formulae did not appear to be affected by diuretics.

Males

Males were divided into two groups according to whether or not diuretics were taken and results are shown in appendix C12. The 36 males who took diuretics during the study tended to be older and less mobile ($p<0.002$) than the 63 males who did not. CCr tended to be reduced in those taking diuretics, with a small difference seen when CCr/SA was compared ($p<0.05$). CCram, CCrpm & CCrn tended to be reduced in the group taking diuretics but this was not significant. The difference between 24h & 8h CCr was not influenced by diuretic taking. The accuracy of CCr prediction using the 5 selected formulae was not altered significantly when diuretics were taken.

3.3.2 Measured CCr vs Predicted CCr

Females

When measured v predicted CCr were plotted for the 5 best fit formulae the accuracy of prediction was seen to alter with magnitude of the measured CCr. If the "ideal" line of measured = predicted CCr was drawn, the point of interception between the two lines occurred between 40 & 50ml/min. Below this point there was a tendency to over-estimate CCr, above this point CCr tended to be under-estimated with the greatest discrepancies seen for extreme values of measured CCr. Subsequently, females were divided into groups of CCr > 50ml/min & CCr < 50ml/min and compared. Results are given in appendix C13.

Those with CCr < 50ml/min were older, less mobile, excreted

less UCr but had a greater SCr. The accuracy of CCr prediction for urine collections less than 24h was not compromised by a reduced CCr, with no statistical difference seen between absolute or percent differences for CCram, CCrpm or CCrn. However, when CCr was predicted using the formulae a significant difference was seen between both the absolute & percent differences of the predicted CCr. CCr tended to be overestimated when measured CCr < 50ml/min and underestimated when measured CCr > 50ml/min.

Males

When measured v predicted CCr was plotted for the males, a similar pattern was seen, with the accuracy of prediction changing with the measured CCr. The intercept between the lines measured v predicted CCr and measured = predicted CCr occurred between 30 & 40ml/min. Although a lower intercept was seen between measured & predicted CCr than for the female group, males were still divided into groups of CCr > 50ml/min & CCr < 50ml/min since it is below this point that renal dysfunction starts to become important in drug dosage calculations (45). Groups were compared with results given in appendix C14.

Those with CCr < 50ml/min were older, less mobile, excreted less UCr & had a higher SCr. CCram & CCrpm remained comparable to 24h CCr for both groups, but CCrn tended to overestimate CCr when CCr was < 50ml/min. When CCr predicted by the formulae were examined a significant difference was seen between both the absolute and percent difference of the predicted CCr. CCr tended to be over-estimated when measured CCr < 50ml/min and underestimated when CCr > 50ml/min.

3.3.3 Influence of Frailty on CCr prediction

Females

Females were divided into two groups of all fit (very fit + fit) & frail subjects and compared with results in appendix C15. The frail group was 6.4y older, less mobile, excreted less UCr (934mg v 639mg), had a greater SCr (1.24mg/dl v 1.63mg/dl) & lower CCr (57ml/min v 36ml/min). The accuracy of CCr prediction was not compromised for urine collections of less than 24h in this group. However, when CCr was predicted using the equations both the absolute & percent differences were significantly different between fit & frail groups. CCr tended to be overestimated in the frail group who had a low measured CCr, and underestimated in the fit group where the greatest measured CCr values were seen. The frail group was significantly older than the fit group and it was not possible to determine whether frailty or increased age was the more important determinant of the reduced CCr seen in the frail group. Very fit, fit & frail female subjects were therefore age matched and compared with results in appendix C16,C17,C18.

When age matched, no statistical differences were seen between the very fit & fit female groups in terms of M.Sc, weight, SA, urine volume, UCr, SCr or CCr. CCrpm & CCrn closely predicted 24h CCr but CCram overestimated 24h CCr for the fit group when compared to the very fit group. For each of the serial collections, the regression coefficient was not less than 0.919 and the gradient of the measured v predicted line closely approached unity. The accuracy of CCr prediction using equations was not significantly different between very fit & fit groups, but the variation in accuracy of prediction was wide. The regression

coefficient varied between 0.889-0.565, and those with the greatest CCr tended to have CCr underpredicted.

When age matched fit & frail female groups were compared, frail subjects were less mobile with a significantly lower urine volume, UCr & CCr. There was no difference between groups for weight, SA or SCr. The accuracy of prediction of CCr for urine collections less than 24h was not compromised in the frail group and there was a good correlation between measured & predicted CCr. However, the accuracy of CCr prediction using equations differed significantly between groups. CCr tended to be underpredicted in the fit group who had the greater measured CCr, and overpredicted in the frail group. The correlation between measured & predicted CCr was less good than for the serial 8h urine collections.

When age matched very fit & frail female groups were compared frailty was associated with a reduced M.Sc, urine output, UCr & CCr, although both groups were similar in weight. Accuracy of CCr prediction was comparable between the groups for urine collections less than 24h when the correlation between measured & predicted CCr was good. However, the accuracy of CCr prediction using the formulae differed between groups, with the very fit group showing a strong tendency towards underprediction & the frail group exhibiting a strong tendency towards overprediction.

Males

Males were divided into two groups of all fit & frail subjects and compared; results are given in appendix C19. The frail group was on average 6.5y older, less mobile, had a lower urine output, excreted less UCr (1371mg v 950mg), had a greater SCr (1.42mg/dl v 2.00mg/dl) and lower CCr

(73ml/min v 44ml/min). The accuracy of CCr prediction for urine collections less than 24h was not compromised for CCram or CCrn although CCrpm tended to overestimate CCr in the fit group ($p<0.05$). However, accuracy of CCr prediction using the 5 best equations varied significantly between groups. CCr tended to be underpredicted in both groups but the degree of underprediction was greater in the fit group who had the greater measured CCr. The frail group was older than the fit group, and in order to separate out the influence of frailty against increased age, very fit, fit & frail males were age matched and compared, with the results given in appendices C20, C21 & C22.

No statistical differences were seen between age matched very fit & fit male groups for M.Sc, weight, SA, urine vol, SCr or CCr, although UCr excretion was lower in the fit group. Accuracy of CCr prediction from reduced urine collections was not significantly different between groups, although CCrpm was the least accurate. CCr predicted from equations tended to be less than the measured CCr for both groups, with the discrepancy between measured & predicted CCr significantly greater for the very fit group.

When age matched fit & frail male groups were compared frail subjects were significantly less mobile, had a lower weight & SA, excreted less UCr, with a lower CCr. Both CCrpm & CCrn accurately predicted CCr for both groups but CCram tended to underpredict CCr in the frail group ($p<0.05$). Using the equations CCr tended to be underpredicted in both groups, with the degree of underprediction greater in the fit group.

When age matched very fit & frail male groups were compared

a similar pattern was seen, although most differences failed to reach significance with only 7 subjects in each group. The frail group was significantly less mobile, weighed less & excreted less UCr than their very fit counterparts, and CCr tended to be reduced in the frail group (76±16ml/min fit, 58±29ml/min frail). The accuracy of CCr prediction using both reduced urine collections and equations was not significantly different between groups although CCr tended to be underpredicted most in the very fit group who had the greater measured CCr.

Fig. 3.1
EQUATIONS FOR THE PREDICTION OF CREATININE CLEARANCE

age - years SCr - mg/100ml weight - kg

EQUATION 1 - COCKCROFT & GAULT, 1976 - (118)

MALES (E1)

$$CCr\ (ml/min) = \frac{(140 - age)\ (weight)}{72 \times SCr}$$

FEMALES (E1F)
CCr males x 0.85

EQUATION 2 - JELLIFFE, 1971 - (119)

MALES (E2)

$$CCr\ (ml/min/1.73m^2) = \frac{100}{SCr} - 12$$

FEMALES (E2F)

$$CCr\ (ml/min/1.73m^2) = \frac{80}{SCr} - 7$$

EQUATION 3 - JELLIFFE, 1973 - (120)

MALES (E3)

$$CCr\ (ml/min/1.73m^2) = \frac{98 - 0.8(age-20)}{SCr}$$

FEMALES (E3F)
CCr males x 0.9

EQUATION 4 - JELLIFFE & JELLIFFE, 1972 - (121)

MALES (E4)

step 1 $E^{ss} = weight\ (29.3 - 0.203\ age)$

step 2 $R = 1.035 - (0.0037 \times SCr)$

step 3 $E_{corr} = E^{ss} \times R = E$

step 4 $CCr\ (ml/min) = \frac{E}{SCr \times 14.4}$

FEMALES (E4F)
step 1 $E_{ss} = weight\ (25.1 - 0.175\ age)$ then as for males

EQUATION 5 - EDWARDS & WHYTE, 1959 - (37)

(E5)

$$CCr\ (ml/min/1.73m^2) = \frac{94.3}{SCr} - 1.8$$

Fig. 3.1 (contd)

EQUATION 6 - WAGNER, 1971 - (122)

MALES (E6)
CCr (ml/min) = 1.96 - 1.19 (log SCr)

FEMALES (E6F)
CCr (ml/min) = 1.85 - 1.18 (log SCr)

EQUATION 7 - ROWE, 1976 - (123)

(E7)
CCr (ml/min/1.73m^2) = 165.57 - 0.80(age)

EQUATION 8 - ROWE, 1976 - (124)

(E8)
CCr (ml/min/1.73m2) = 133 - 0.64 (age)

EQUATION 9 - MAWER, 1976 - (125)

MALES (E9)
step 1 Q_{cr} = weight (29.3 - (0.203 x age))

step 2 $Q_{cr\ corr} = Q_{cr}$ (1 - (0.003 SCr))

step 3 $CCr\ (ml/min) = \frac{Qcr}{14.4 \times SCr}$

FEMALES (E9F)
$Q_{cr} = Q_{cr}$ males x 0.8

EQUATION 10 - HULL, 1981 - (126)

MALES (E10)
$CCr\ (ml/min/70kg) = \frac{(145 - age)}{SCr} - 3$

FEMALES (E10F)
CCr (ml/min/70kg) = CCr male x 0.85

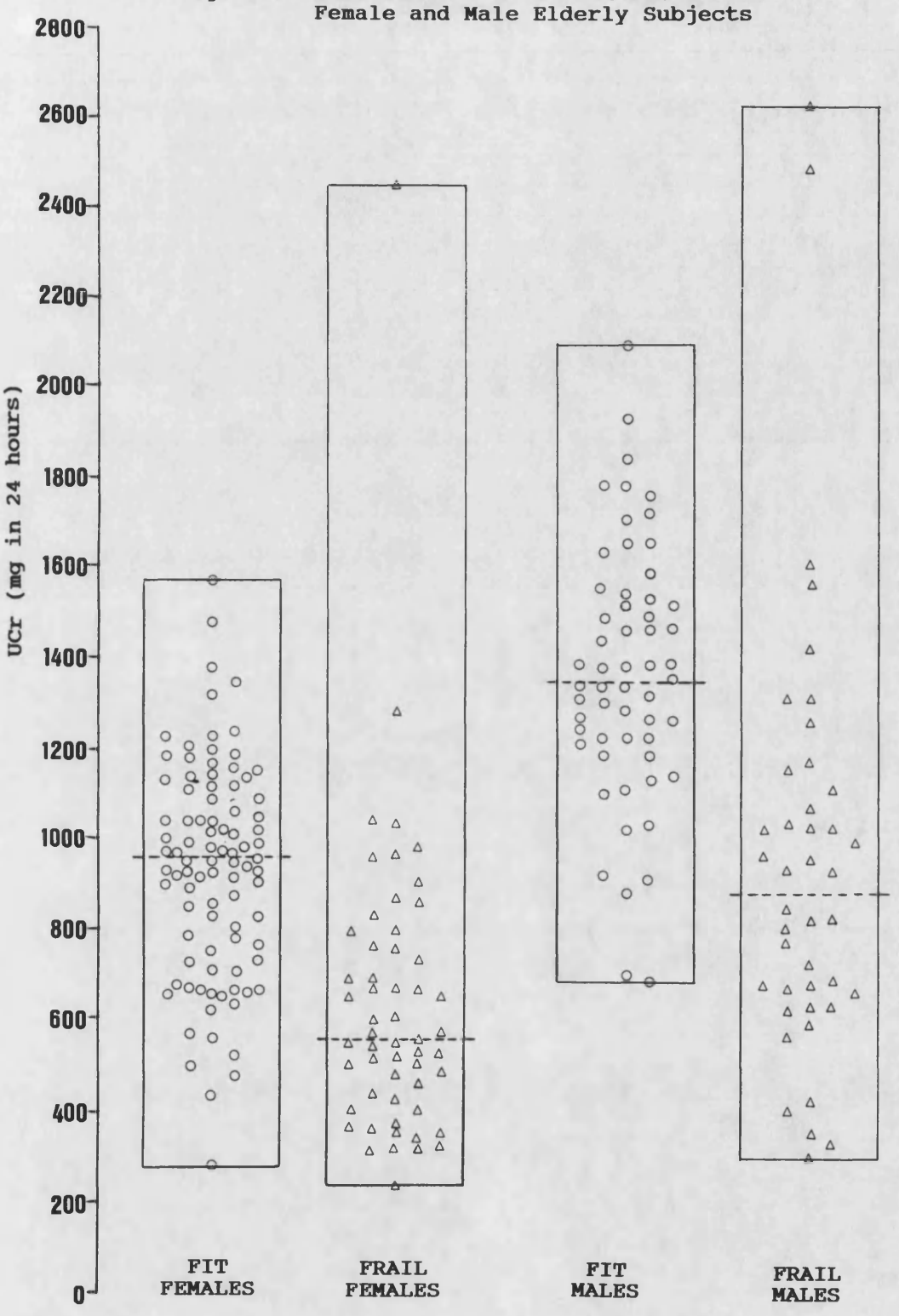
EQUATION 11 - GATES, 1985 - (127)

MALES (E11)
$CCr\ (ml/min) = 89.4\ (SCr^{-1.2}) + (55-age)[0.005(89.4)(SCr^{-1.1})]$

FEMALES (E11)
$CCr\ (ml/min) = 60\ (SCr^{-1.1}) + (56-age)[0.005(60)(SCr^{-1.1})]$

NOMOGRAM 1 (E12) - SIERSBAEK-NIELSEN, 1971 - (129)

Fig. 3.2 Distribution of UCr in Fit & Frail Female and Male Elderly Subjects



○ = fit subject △= frail subject ----median

Fig. 3.3 Distribution of SCr in Fit & Frail Female and Male Elderly Subjects

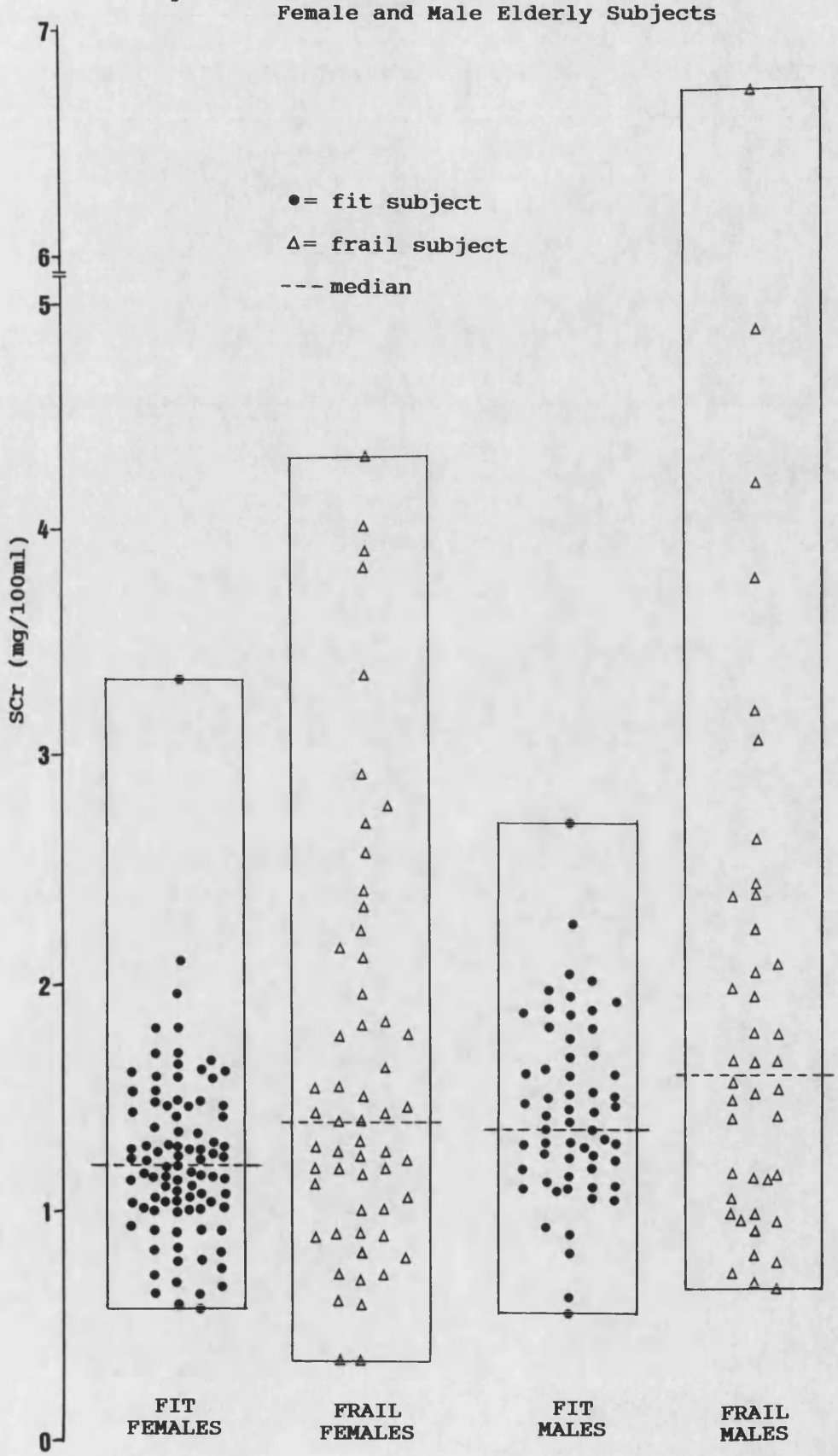


Fig. 3.4 Distribution of CCr/SA in Fit & Frail Female and Male Elderly Subjects

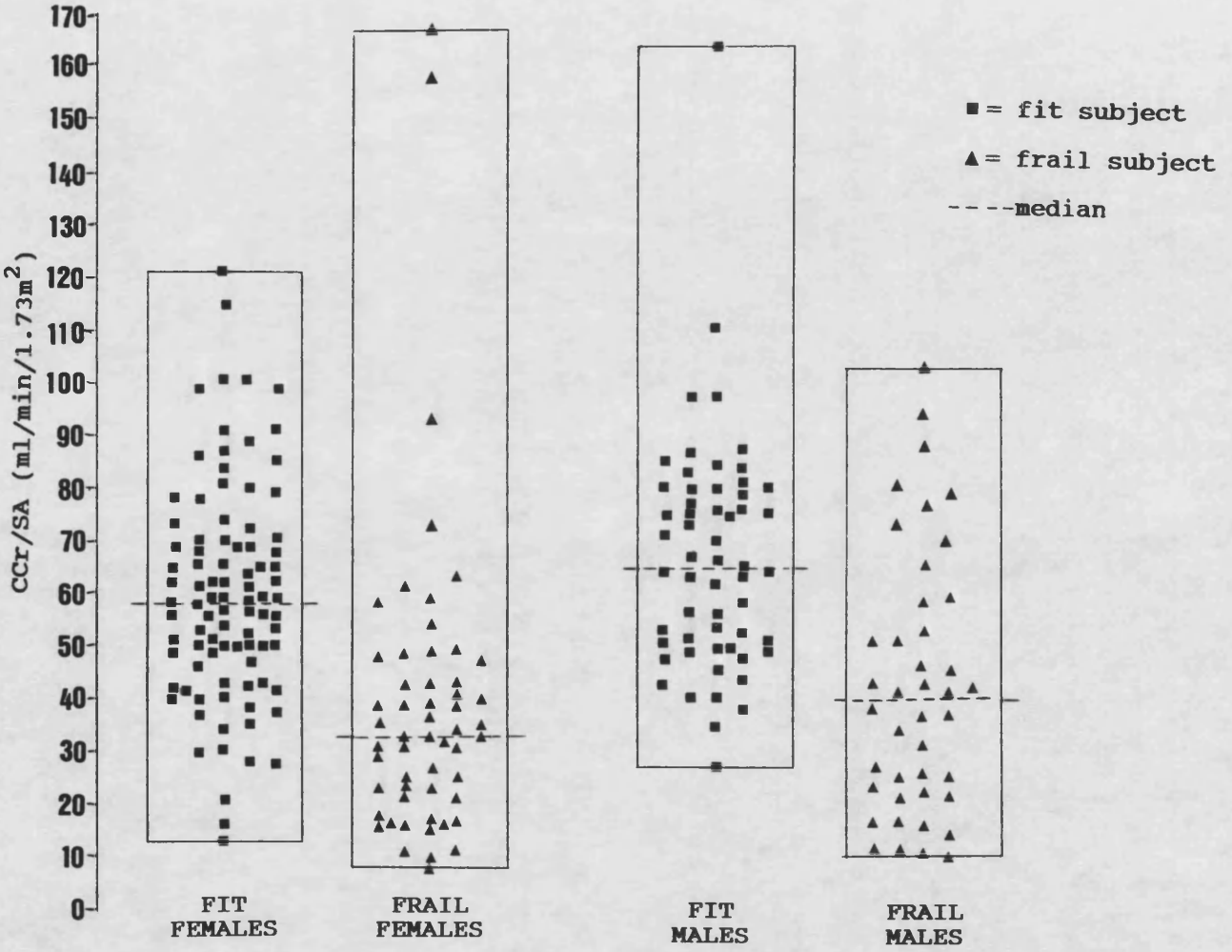


Fig. 3.3.1 Predicted CCram v Measured 24h CCr for Female Subjects

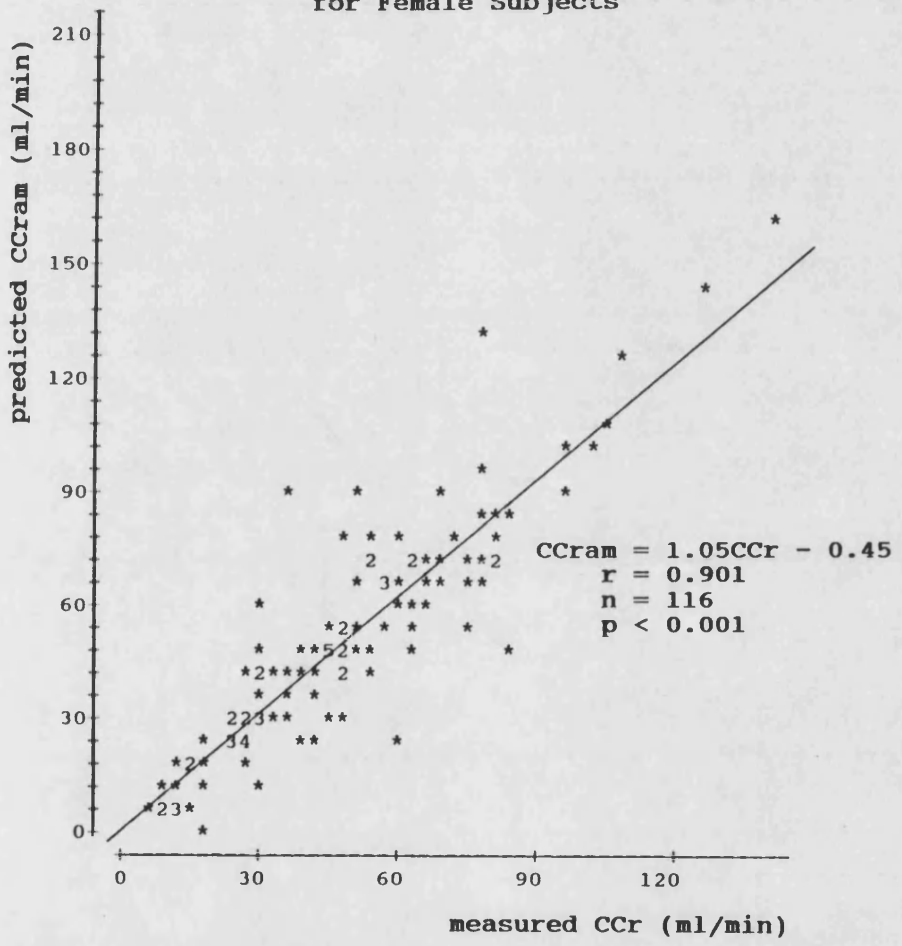


Fig. 3.3.2 Predicted CCrpm v Measured 24h CCr for Female Subjects

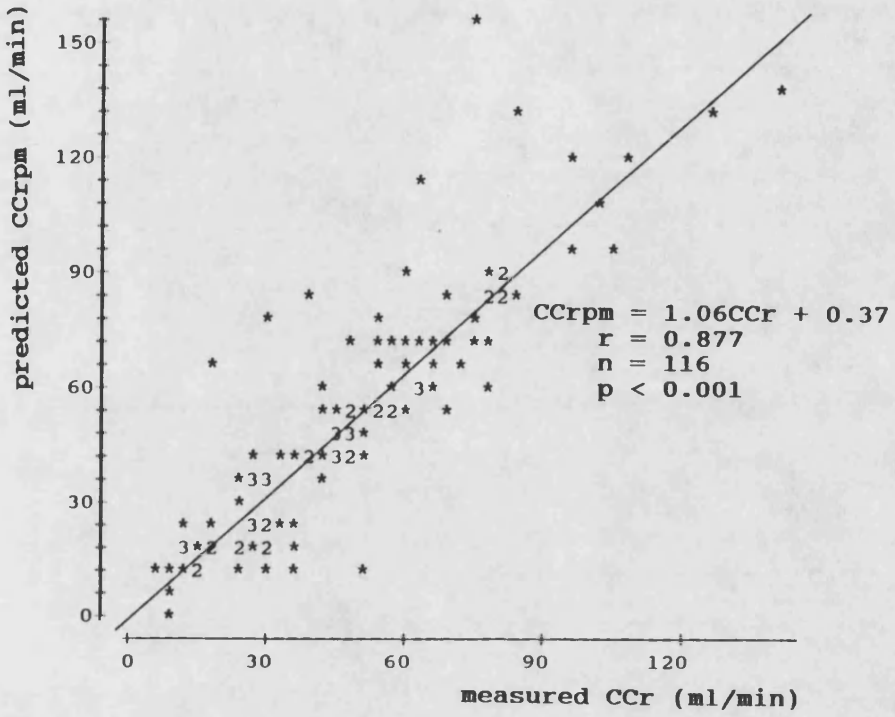


Fig. 3.3.3 Predicted CCrn v Measured 24h CCr for Female Subjects

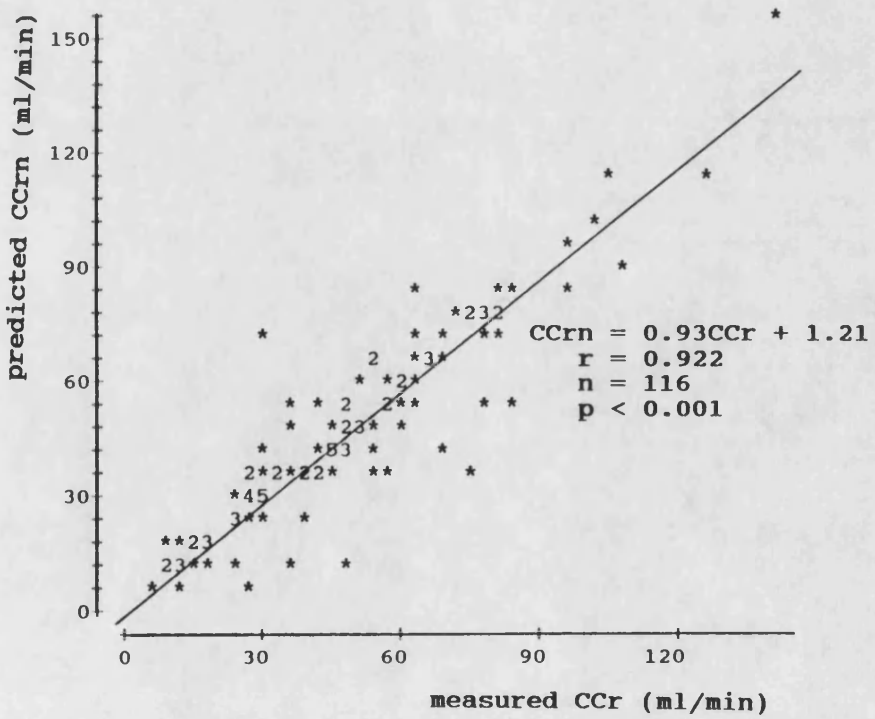


Fig. 3.3.4 Predicted v Measured CCr using E1 for Female Subjects

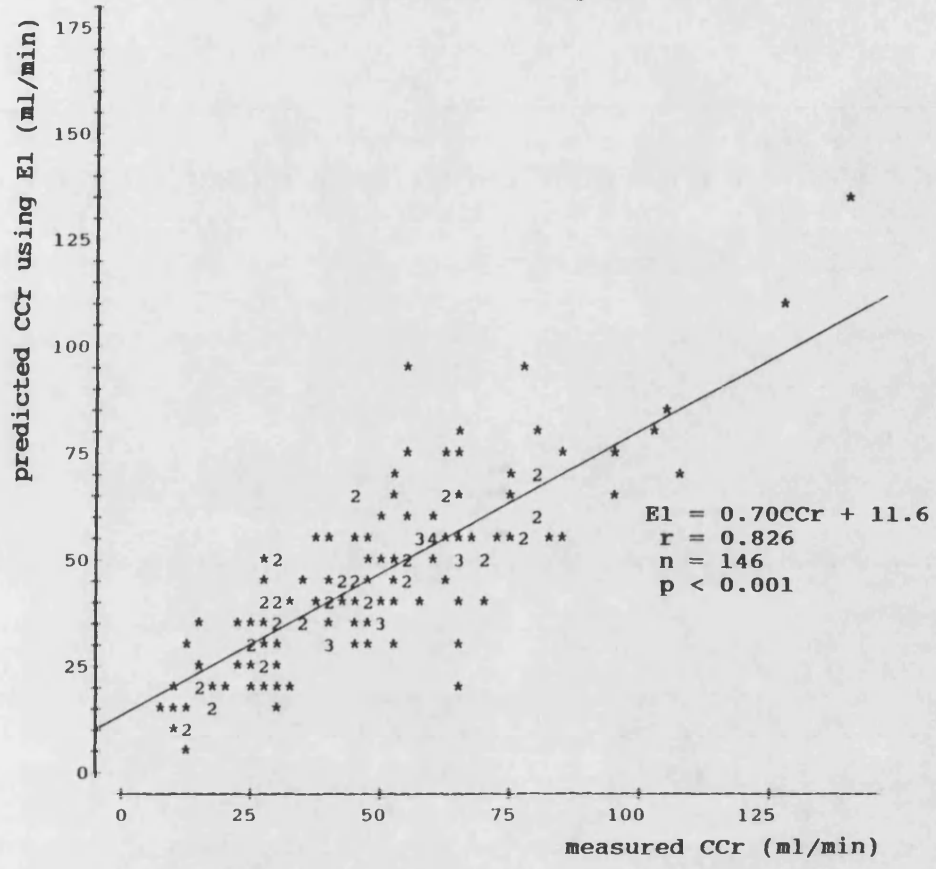


Fig. 3.3.5 Predicted v Measured CCr using E1F for Female Subjects

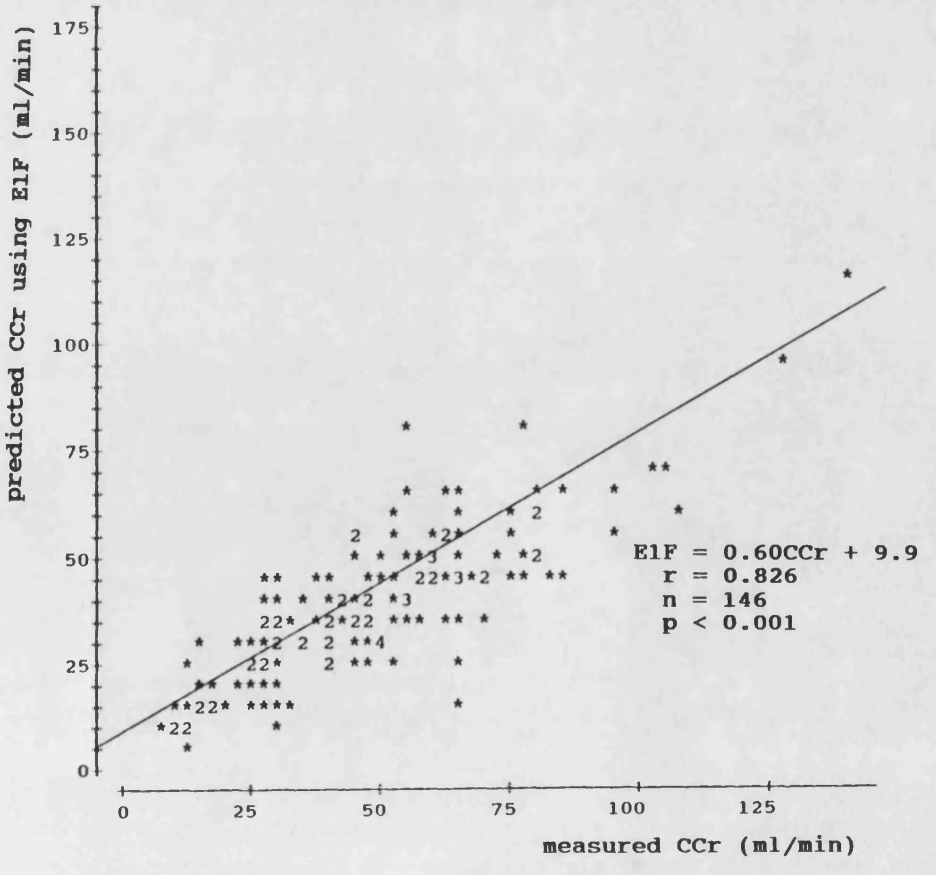


Fig. 3.3.6 Predicted v Measured CCr using E2 for Female Subjects

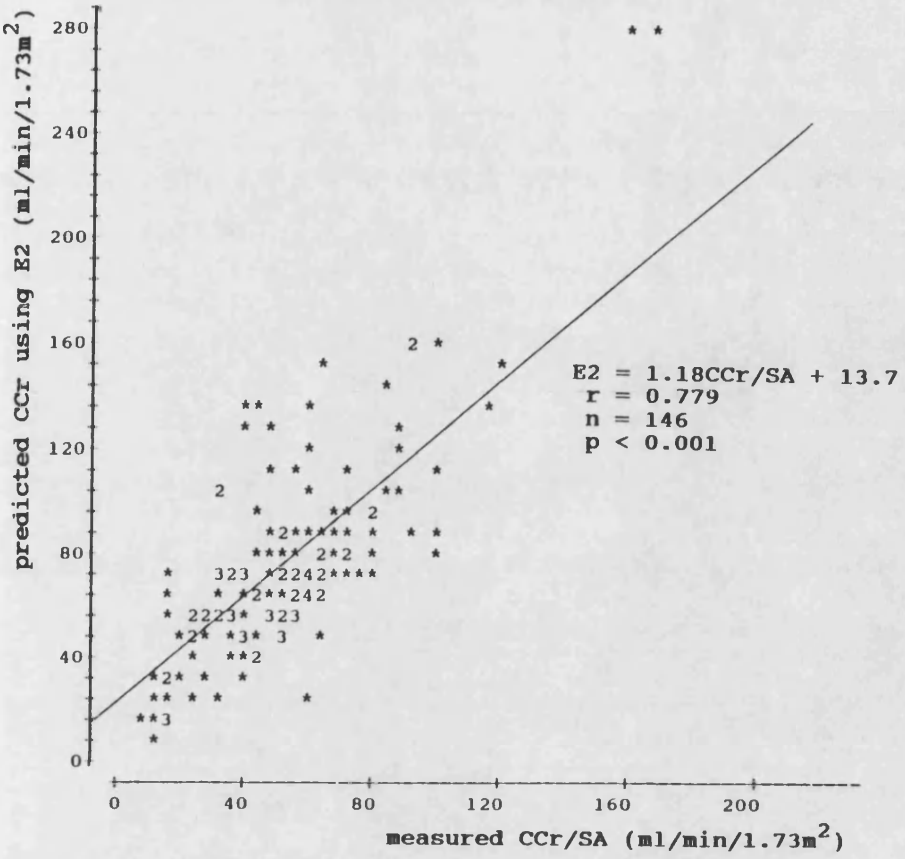


Fig. 3.3.7 Predicted v Measured CCr using E2F for Female Subjects

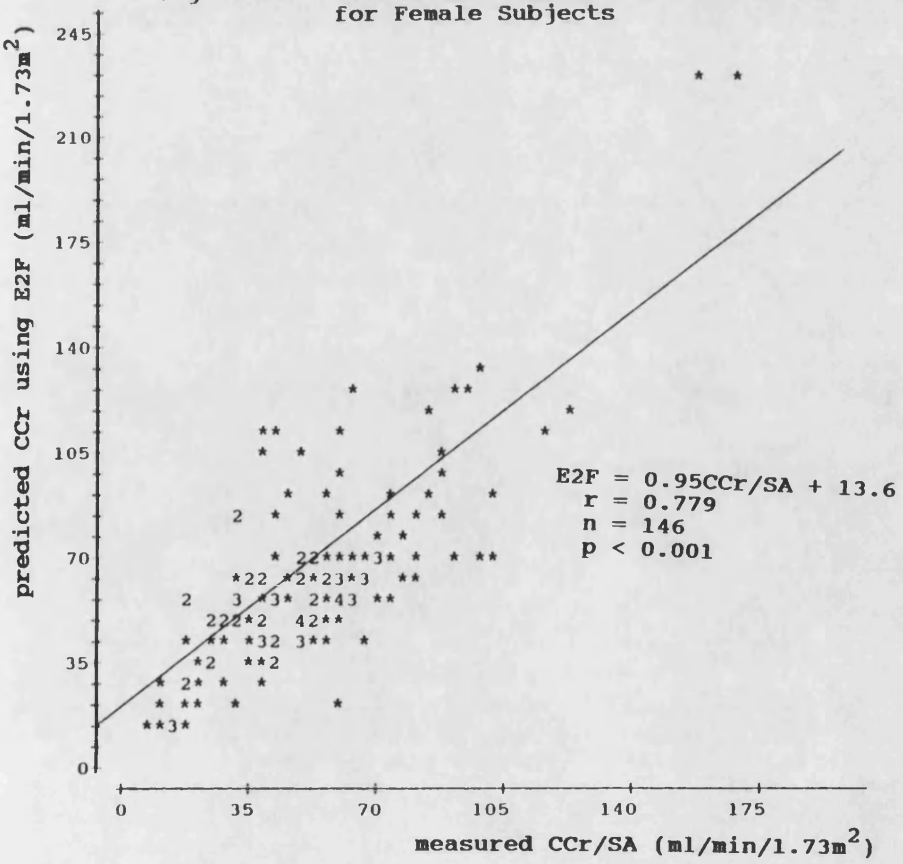


Fig. 3.3.8 Predicted v Measured CCr using E3 for Female Subjects

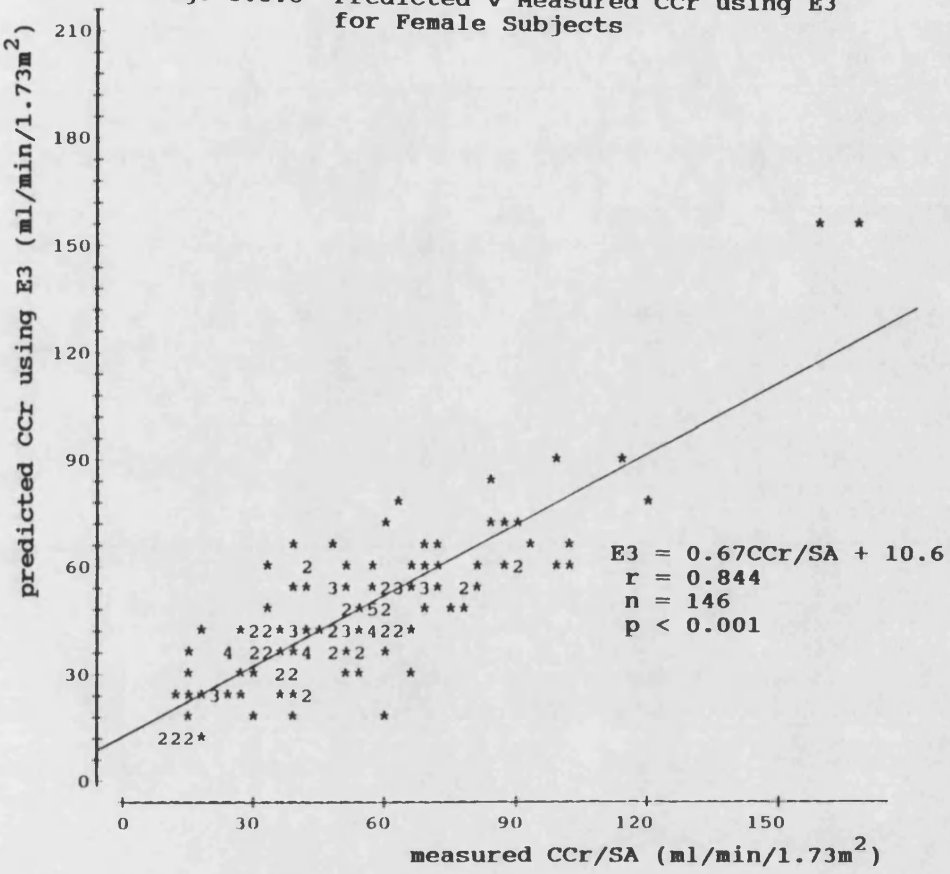


Fig. 3.3.9 Predicted v Measured CCr using E3F for Female Subjects

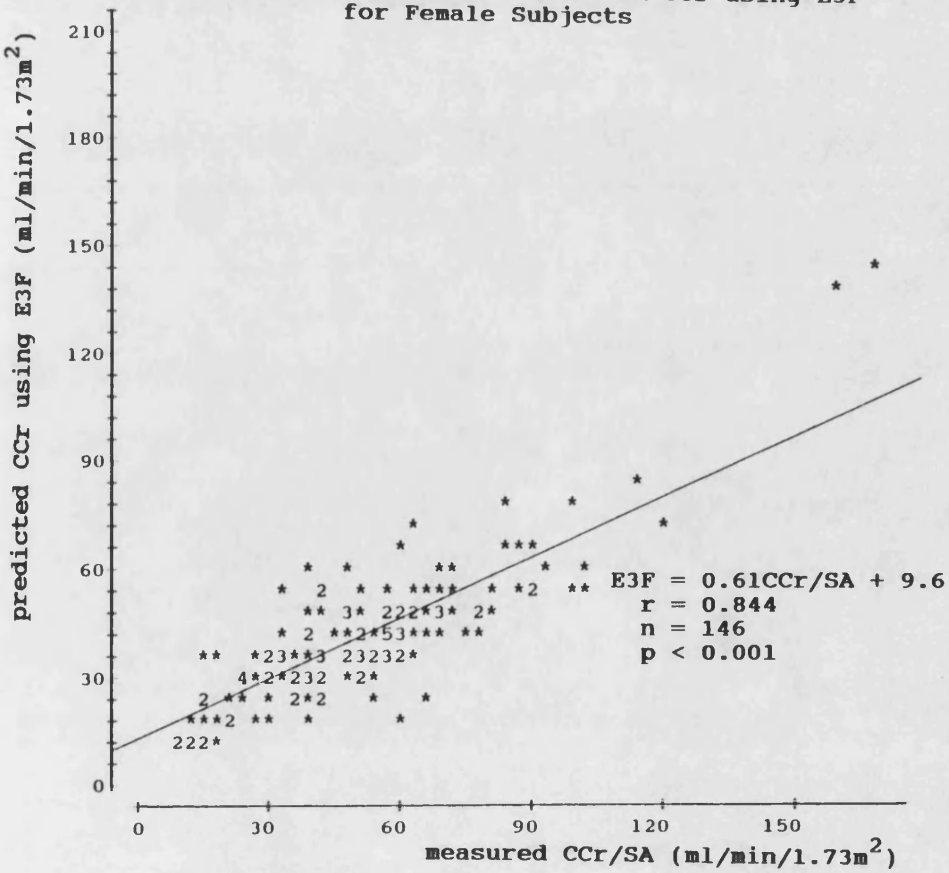


Fig. 3.3.10 Predicted v Measured CCr using E4 for Female Subjects

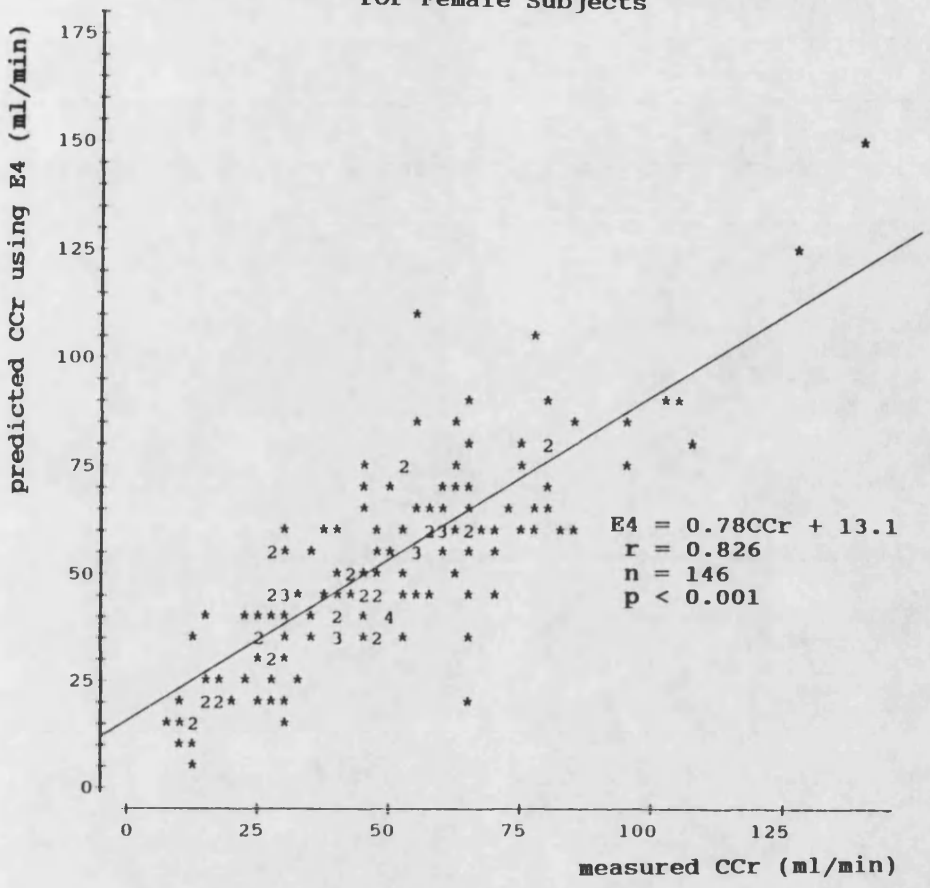


Fig. 3.3.11 Predicted v Measured CCr using E4F for Female Subjects

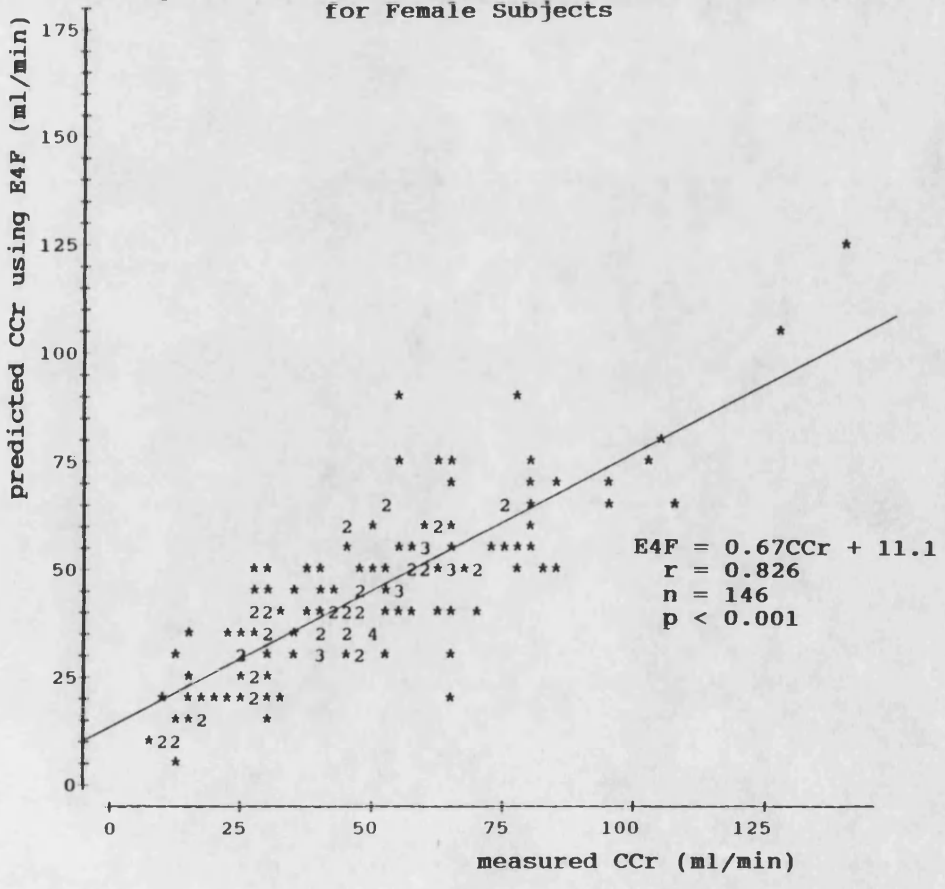


Fig. 3.3.12 Predicted v Measured CCr using E5 for Female Subjects

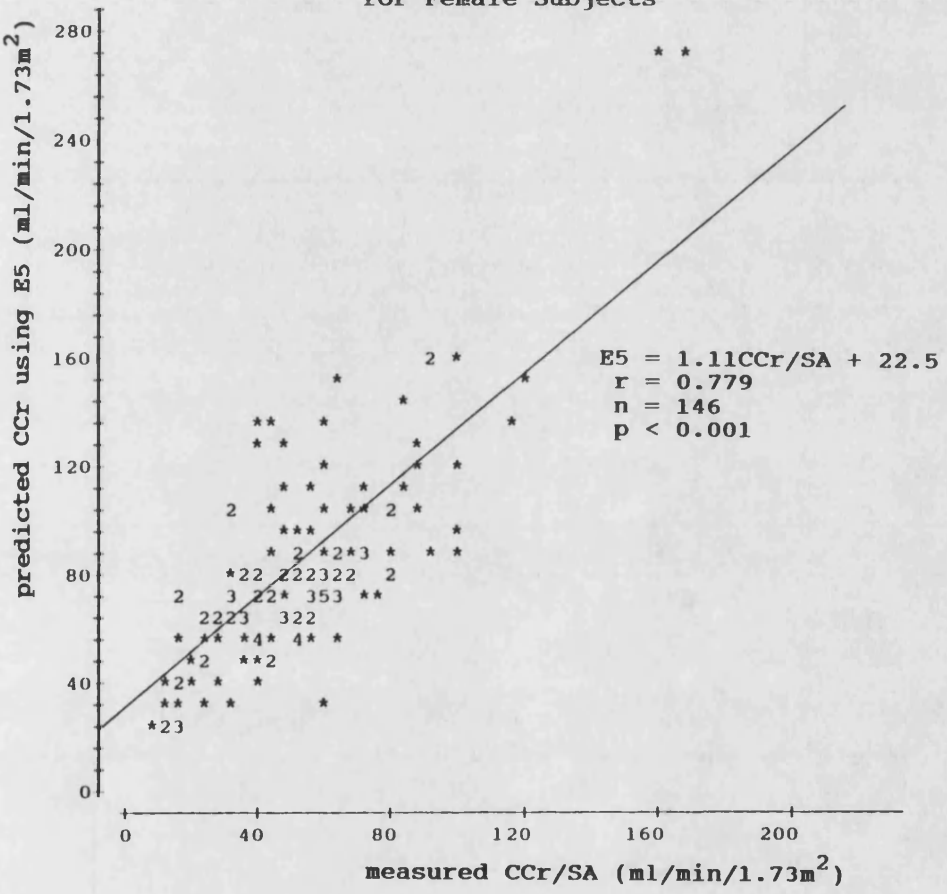


Fig. 3.3.13 Predicted v Measured CCr using E6 for Female Subjects

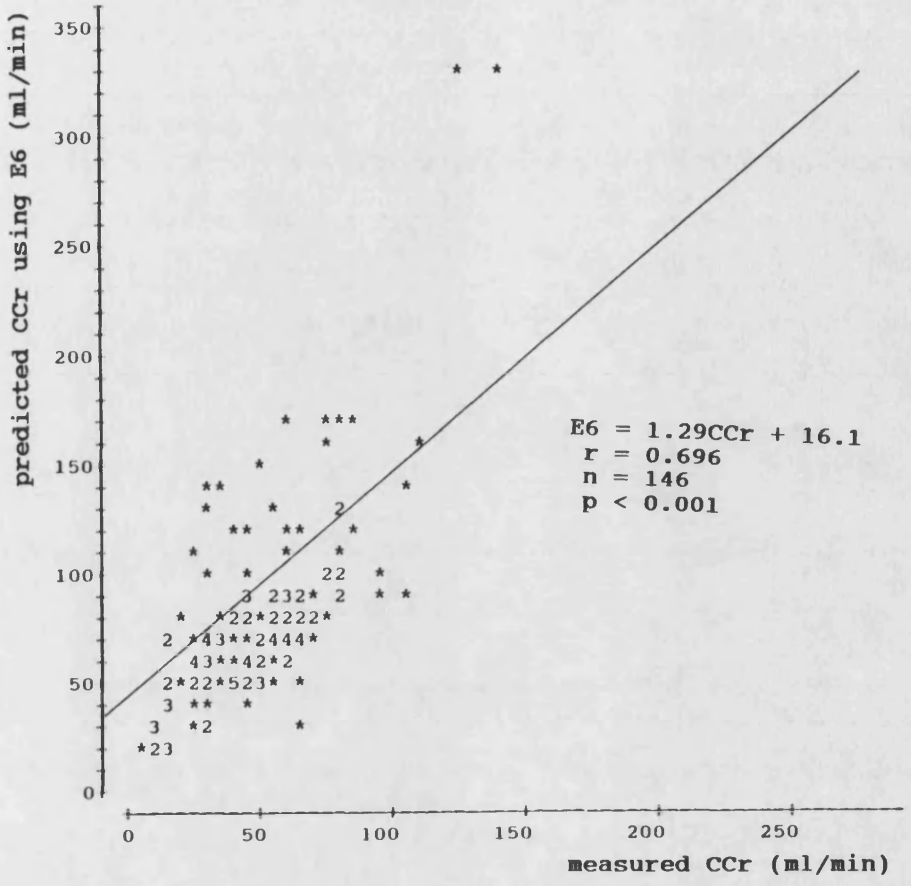


Fig. 3.3.14 Predicted v Measured CCr using E6F for Female Subjects

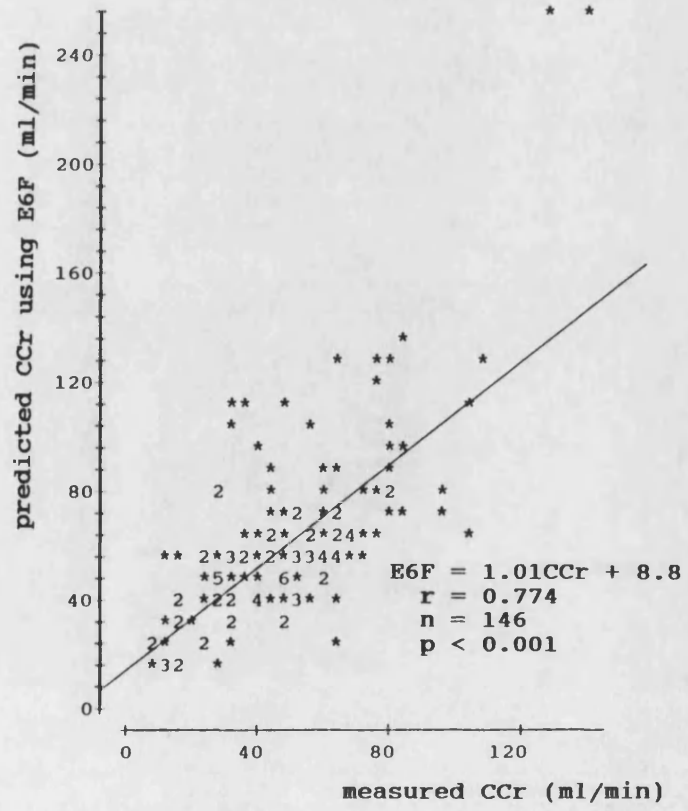


Fig. 3.3.15 Predicted v Measured CCr using E7 for Female Subjects

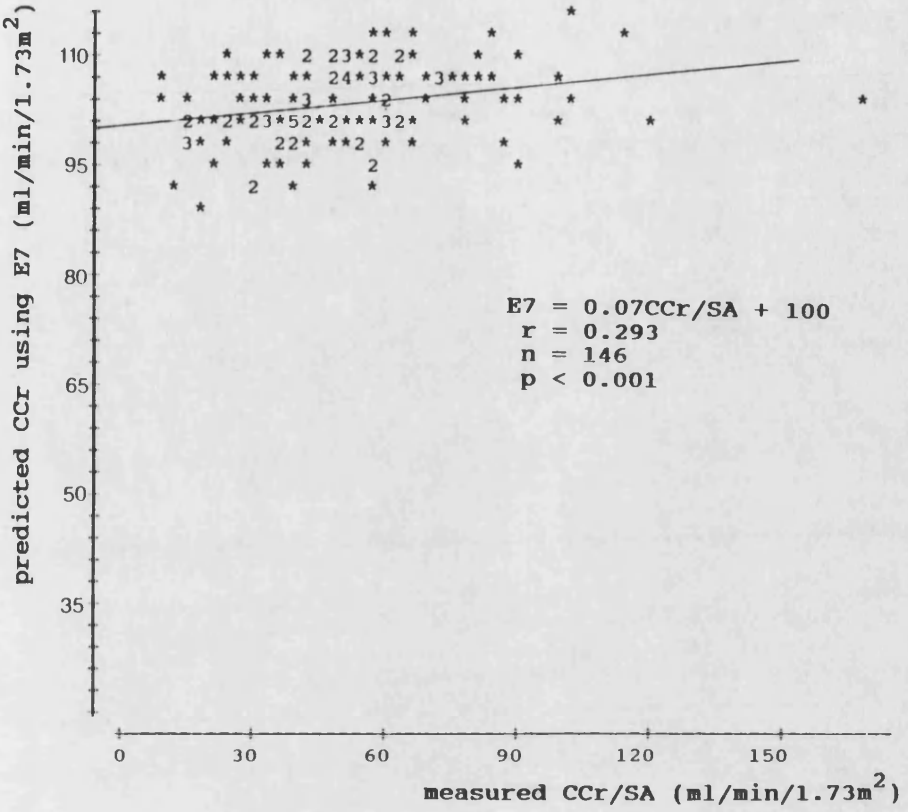


Fig. 3.3.16 Predicted v Measured CCr using E8 for Female Subjects

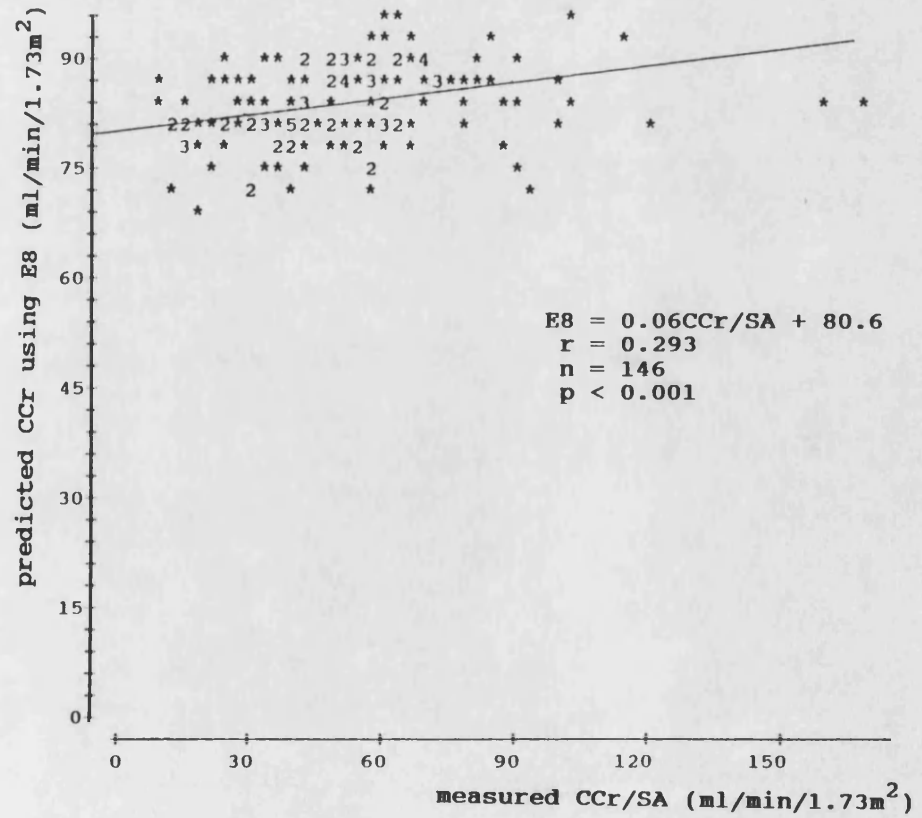


Fig. 3.3.17 Predicted v Measured CCr using E9 for Female Subjects

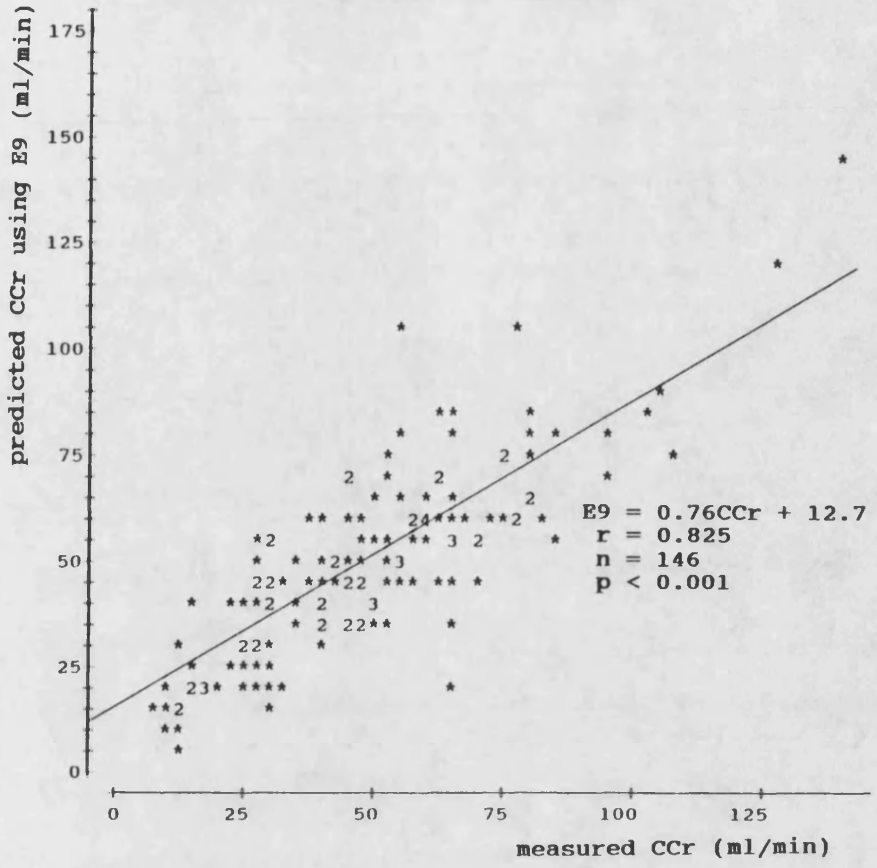


Fig. 3.3.18 Predicted v Measured CCr using E9F for Female Subjects

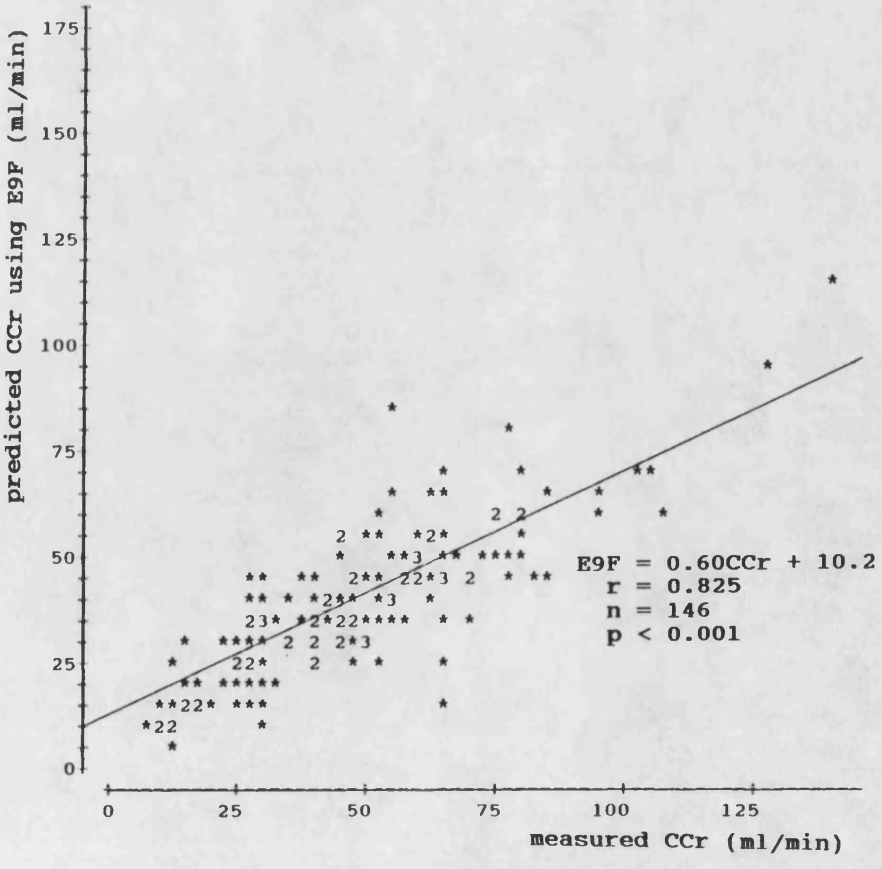


Fig. 3.3.19 Predicted v Measured CCr using E10 for Female Subjects

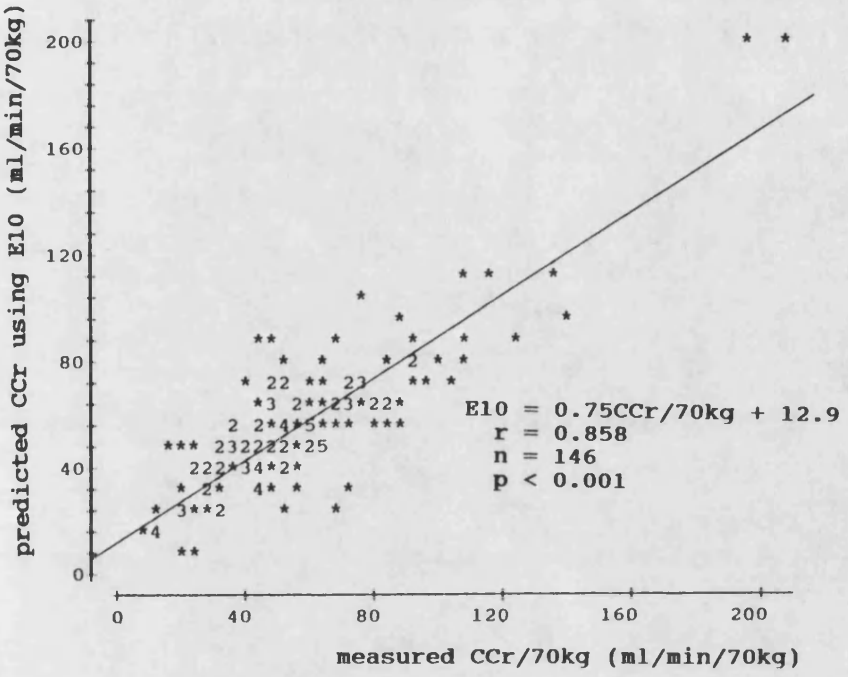


Fig. 3.3.20 Predicted v Measured CCr using E10F for Female Subjects

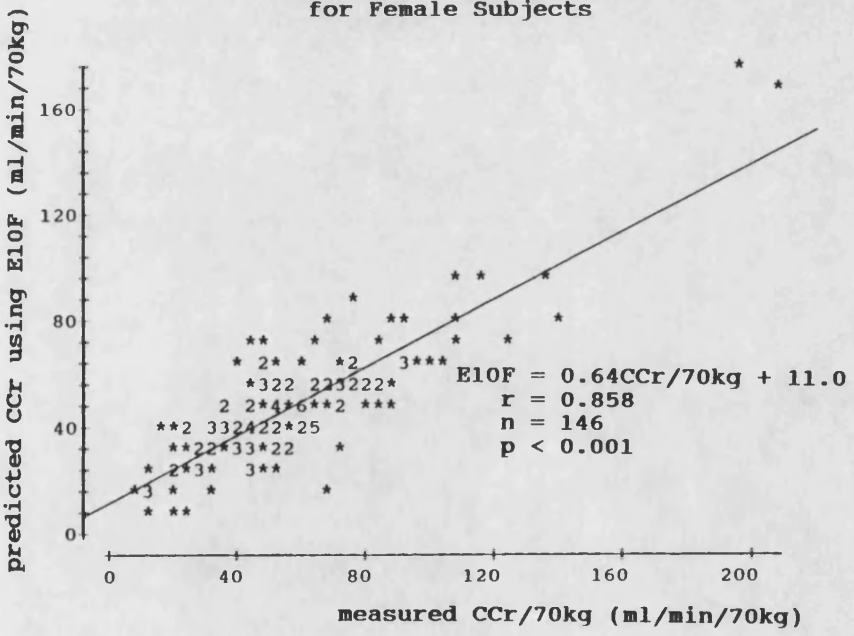


Fig. 3.3.21 Predicted v Measured CCr using E11 for Female Subjects

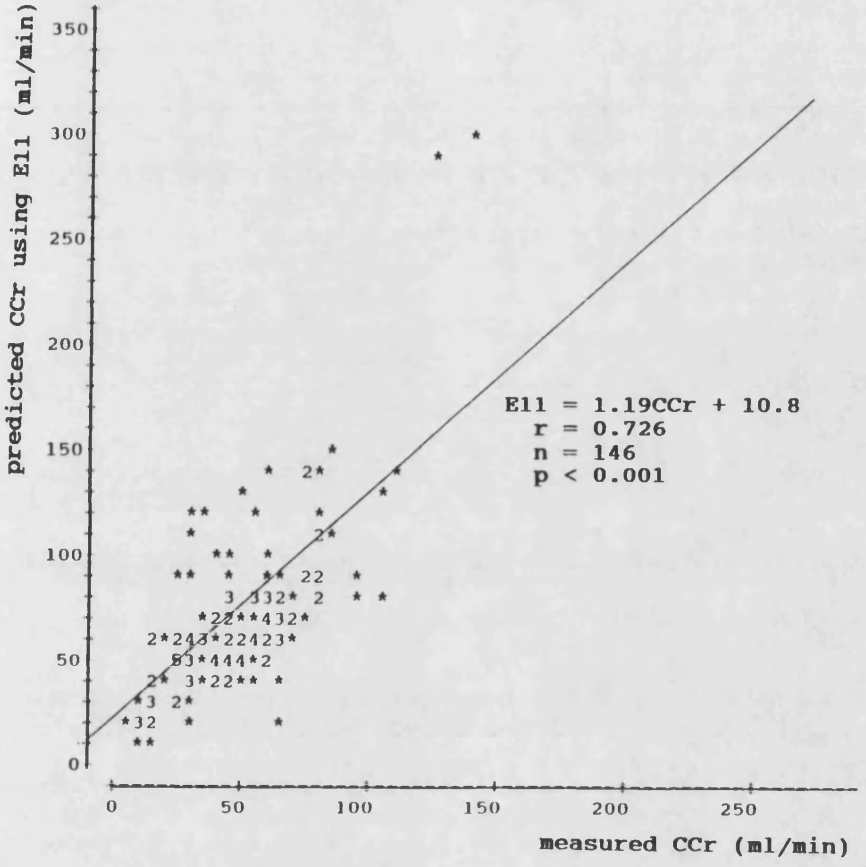


Fig. 3.3.22 Predicted v Measured CCr using E11F for Female Subjects

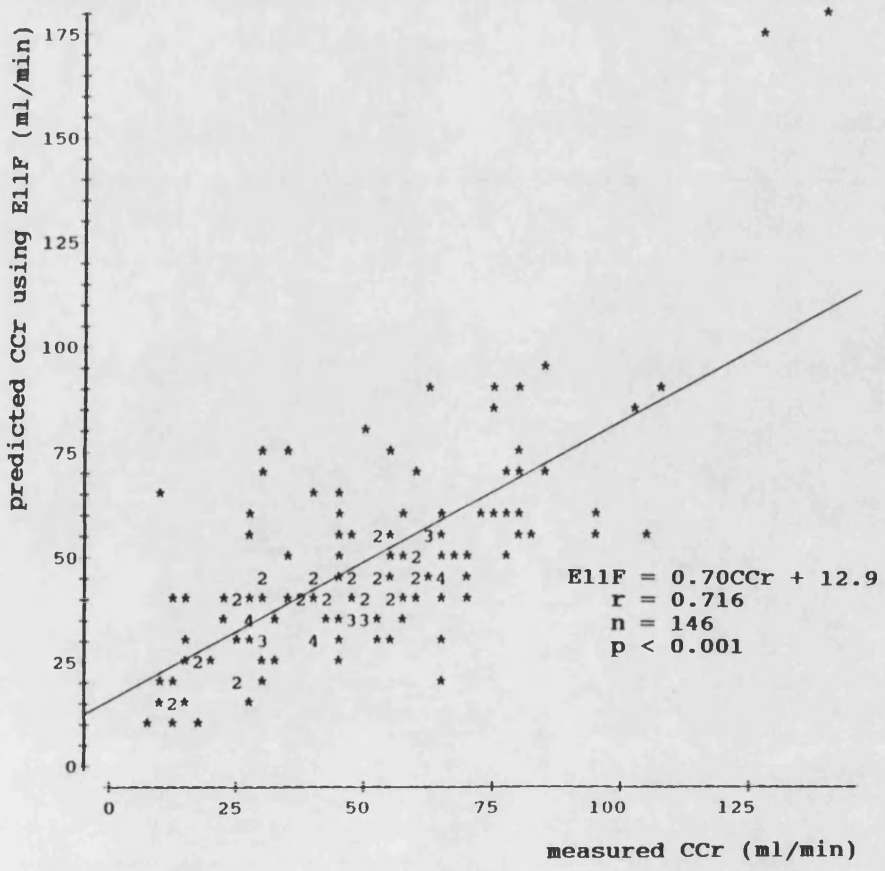


Fig. 3.3.23 Predicted v Measured CCr using E12 for Female Subjects

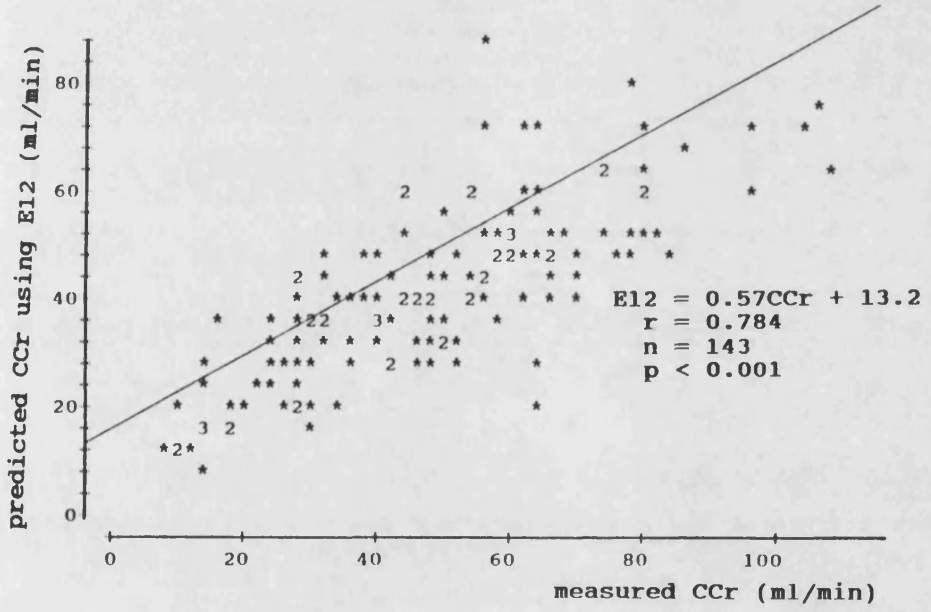


Fig. 3.3.24 Measured 24h CCr v % Predicted CCram for Female Subjects

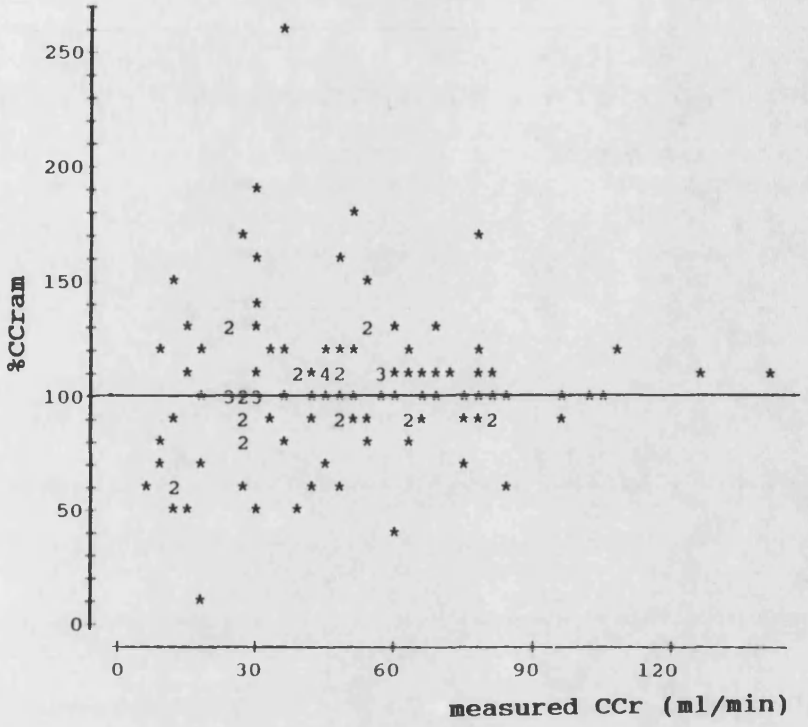


Fig. 3.3.25 Measured 24h CCr v % Predicted CCrpm for Female Subjects

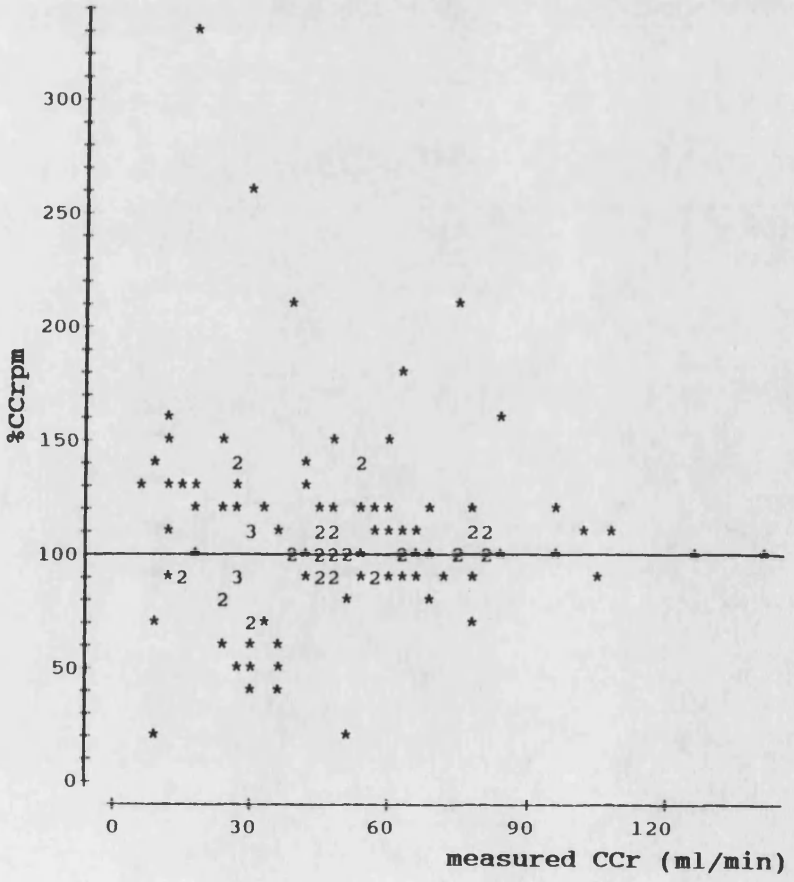


Fig. 3.3.26 Measured 24h CCr v % Predicted CCrn for Female Subjects

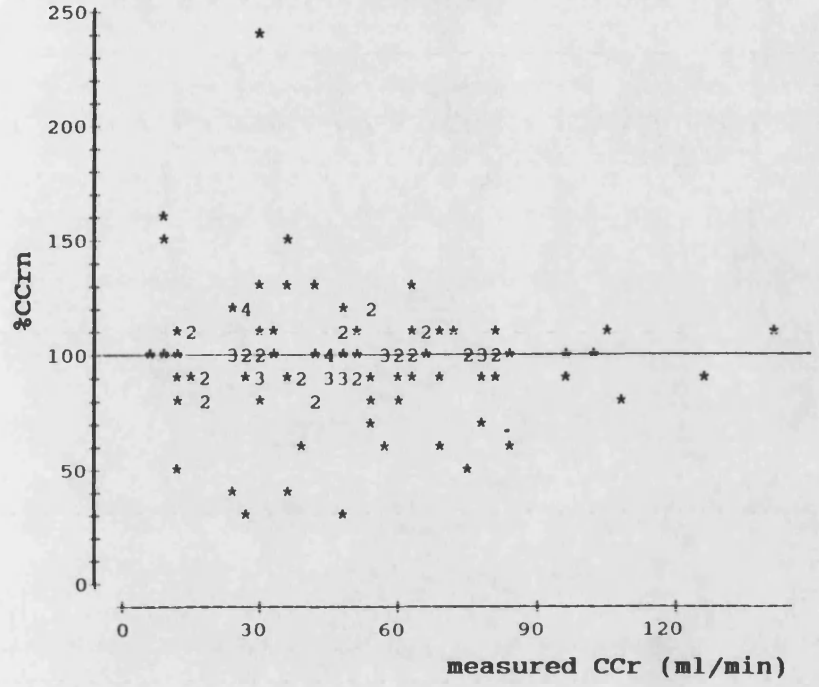


Fig. 3.3.27 Measured 24h CCr v % Predicted CCr Using E1 for Female Subjects

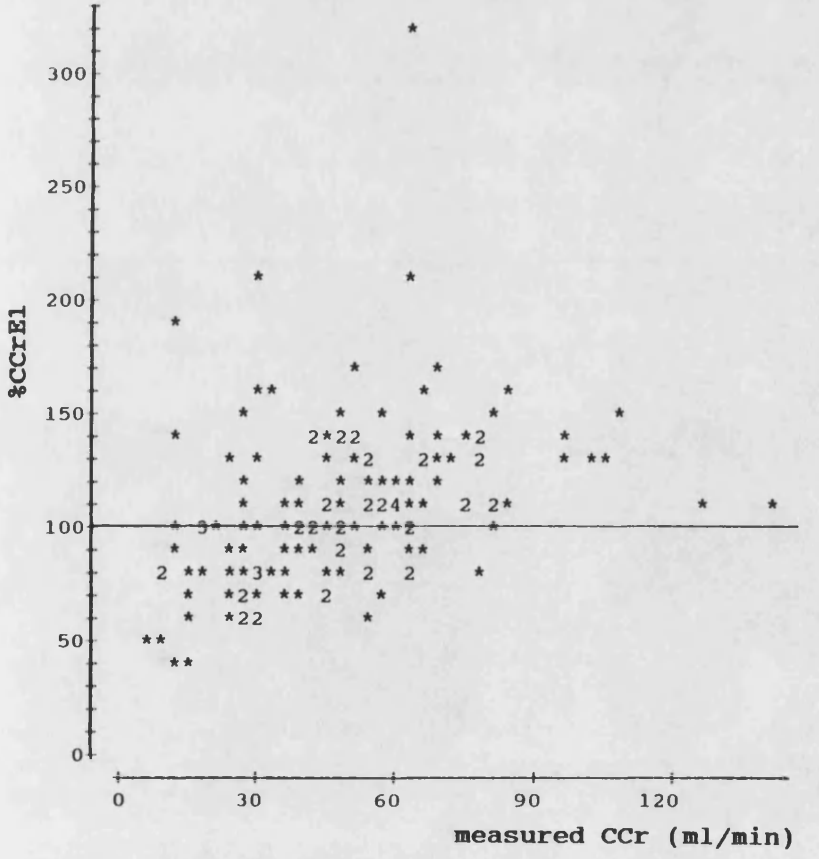


Fig. 3.3.28 Measured 24h CCr v % Predicted CCr Using E4 for Female Subjects

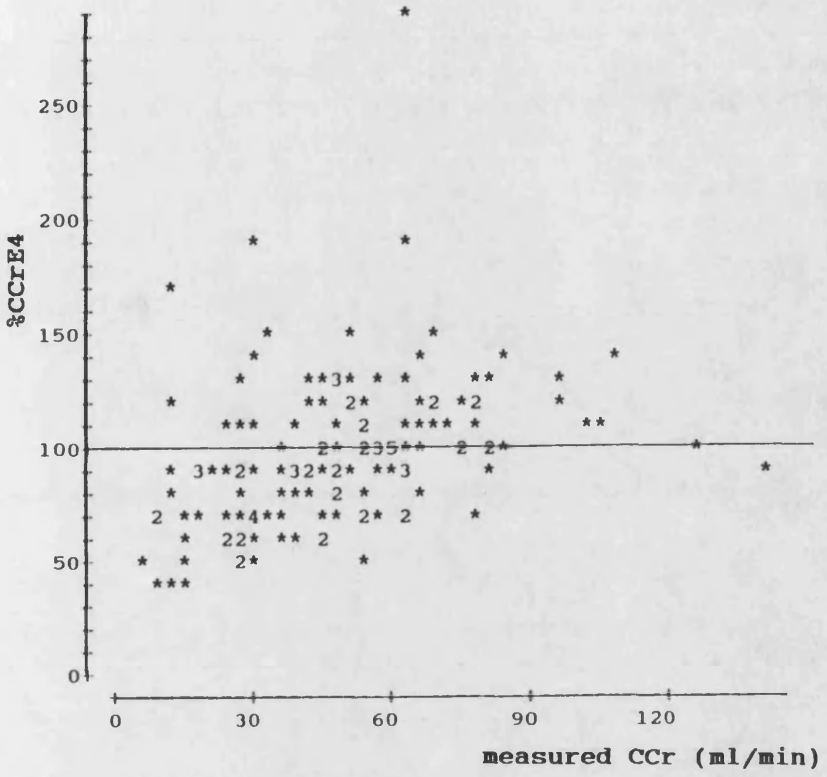


Fig. 3.3.29 Measured 24h CCr v % Predicted CCr Using E9 for Female Subjects

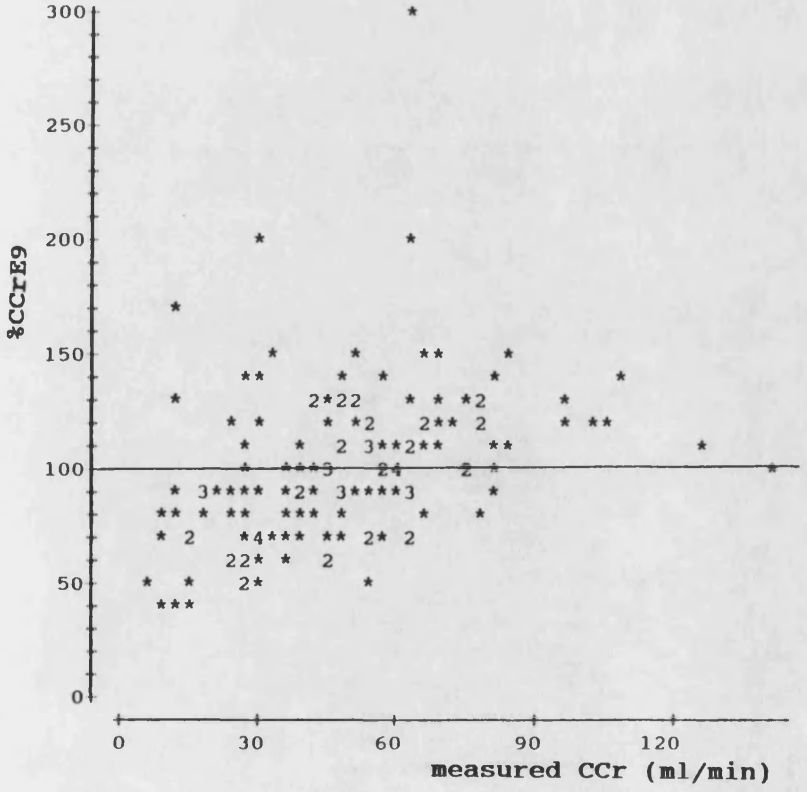


Fig. 3.3.30 Measured 24h CCr v % Predicted CCr Using E10 for Female Subjects

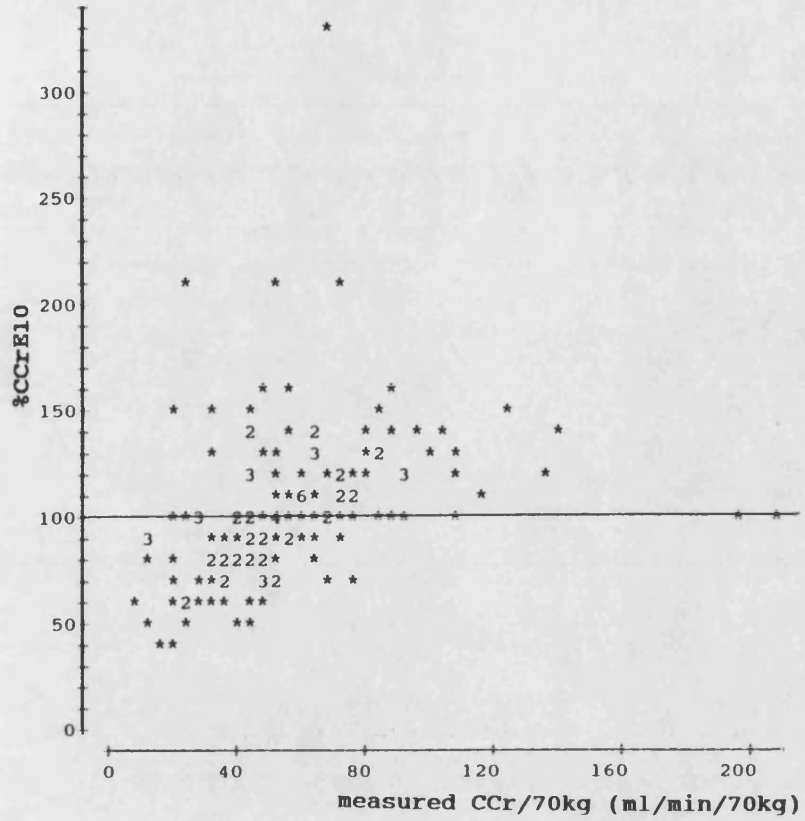


Fig. 3.3.31 Measured 24h CCr v % Predicted CCr Using E11F for Female Subjects

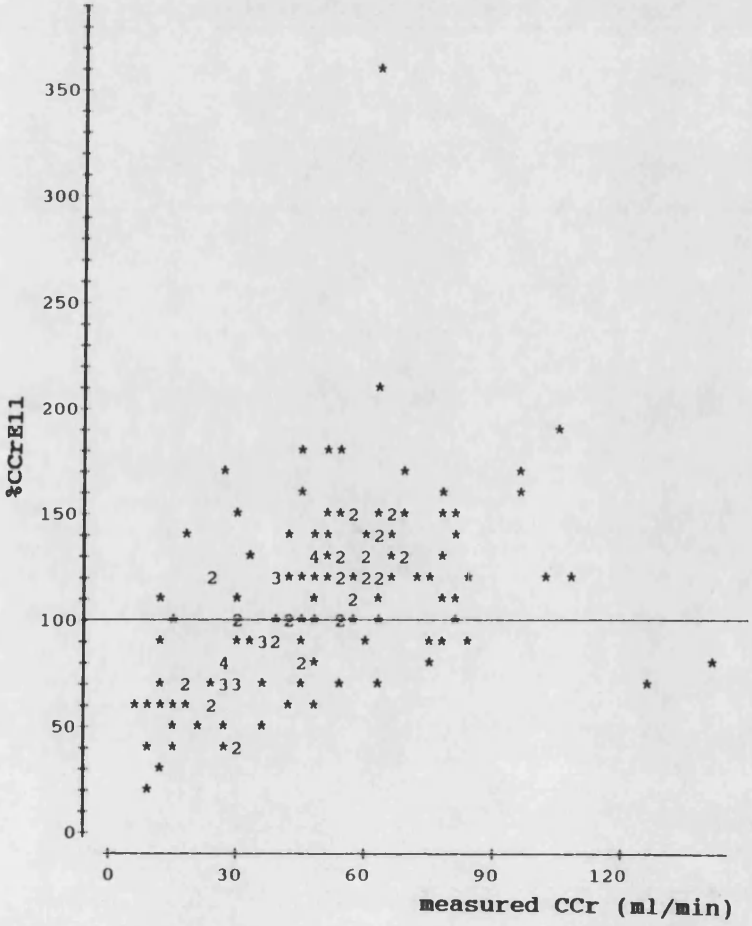


Fig. 3.4.1 Predicted CCram v Measured 24h CCr for Male Subjects

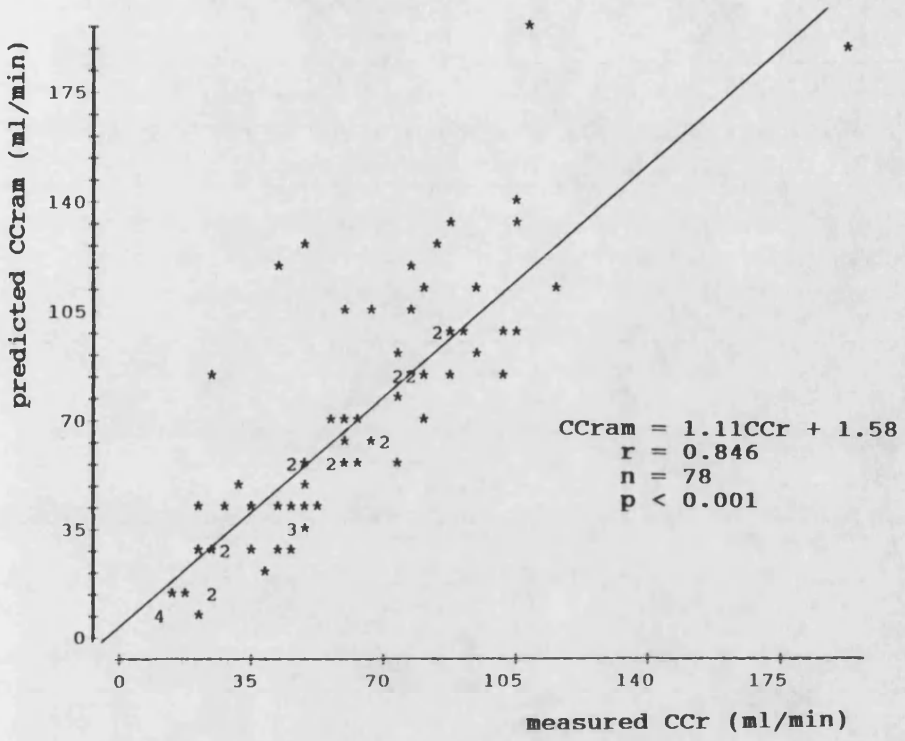


Fig. 3.4.2 Predicted CCrpm v Measured 24h CCr for Male Subjects

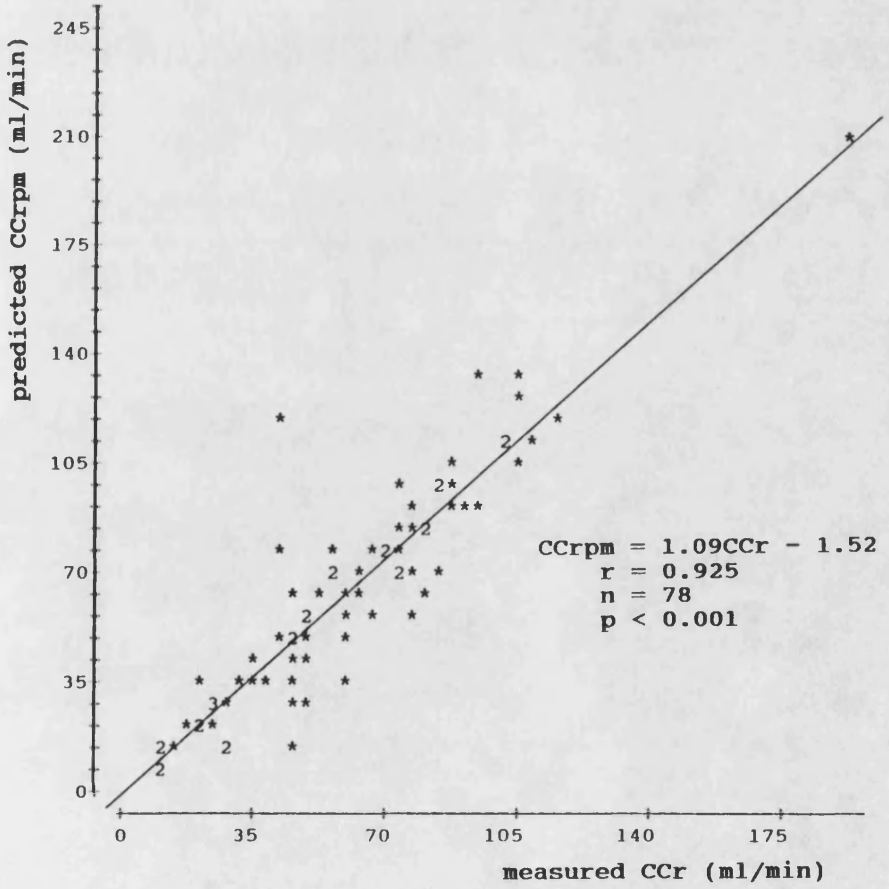


Fig. 3.4.3 Predicted CCrn v Measured 24h CCr for Male Subjects

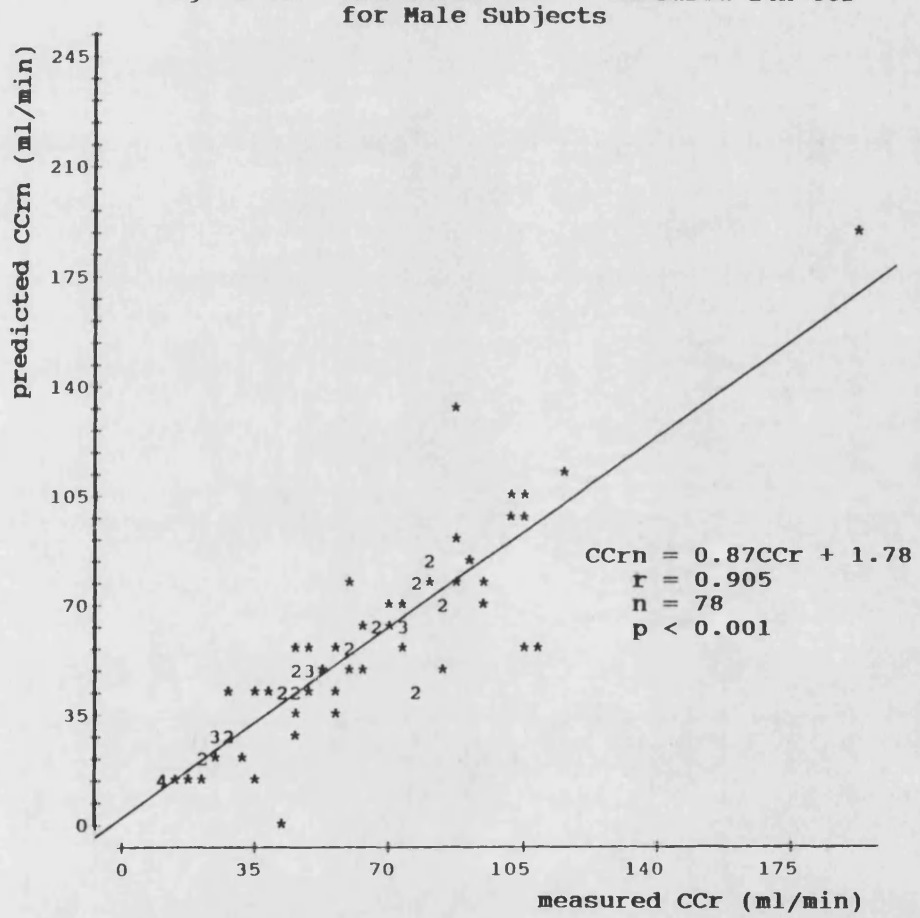


Fig. 3.4.4 Predicted v Measured CCr using E1 for Male Subjects

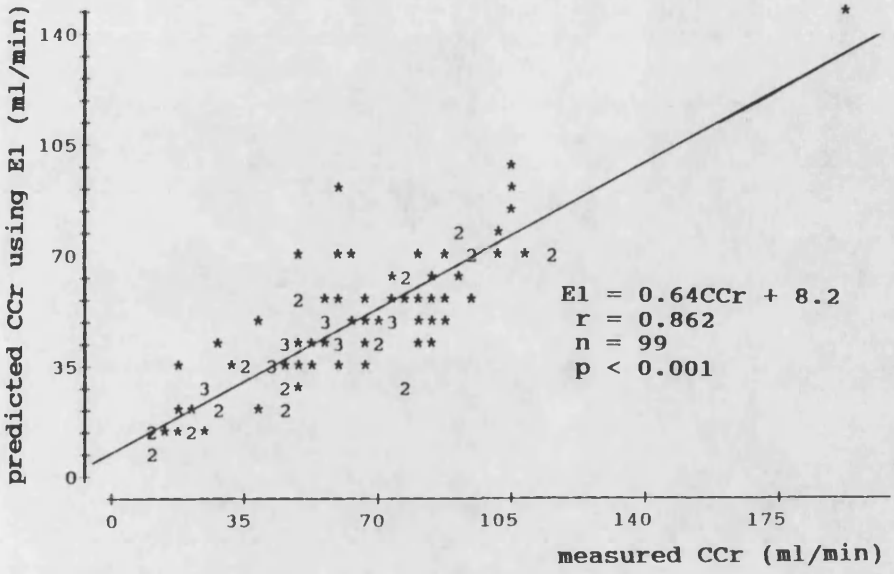


Fig. 3.4.5 Predicted v Measured CCr using E2 for Male Subjects

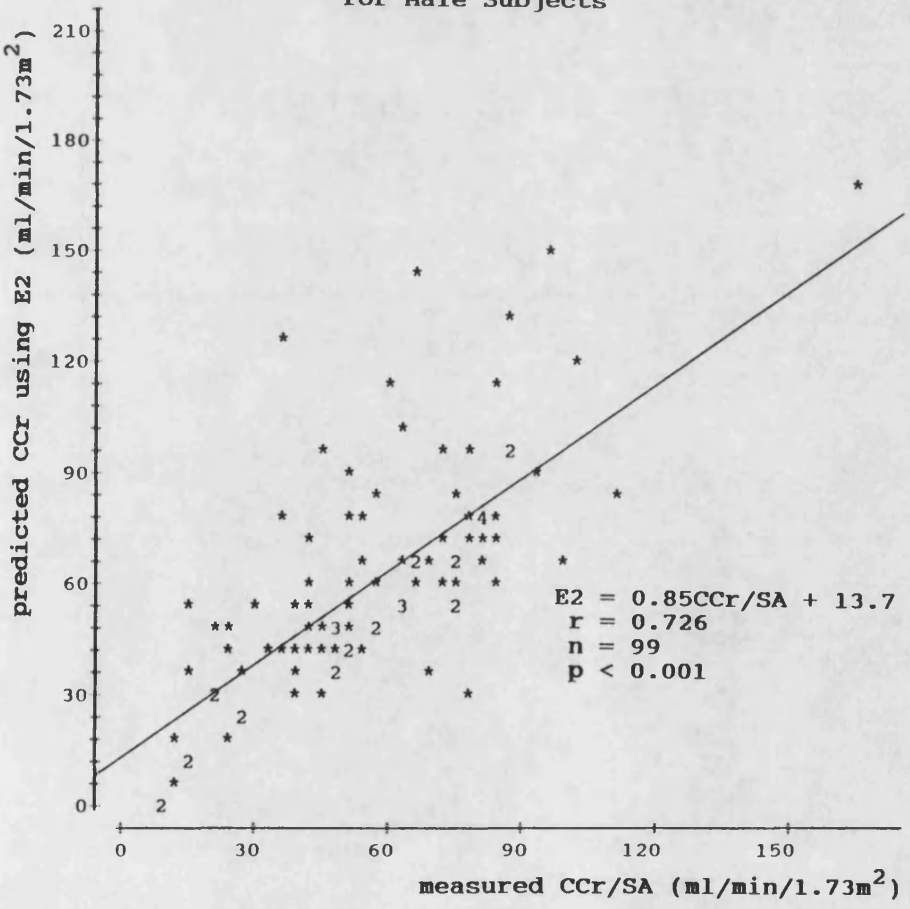


Fig. 3.4.6 Predicted v Measured CCr using E3 for Male Subjects

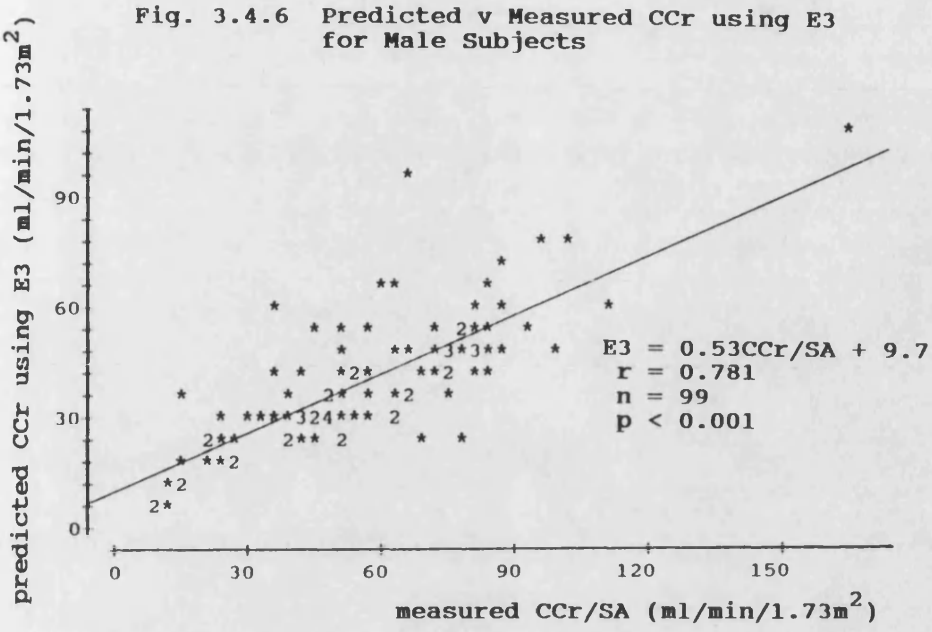


Fig. 3.4.7 Predicted v Measured CCr using E4 for Male Subjects

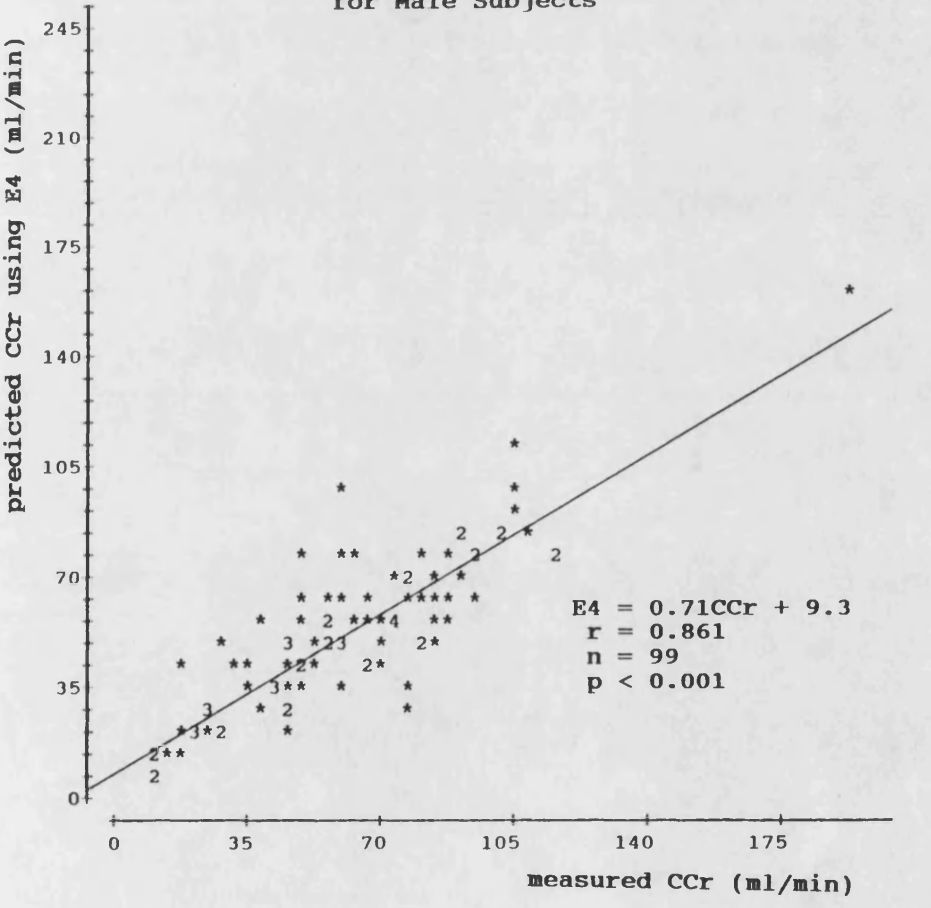


Fig. 3.4.8 Predicted v Measured CCr using E5 for Male Subjects

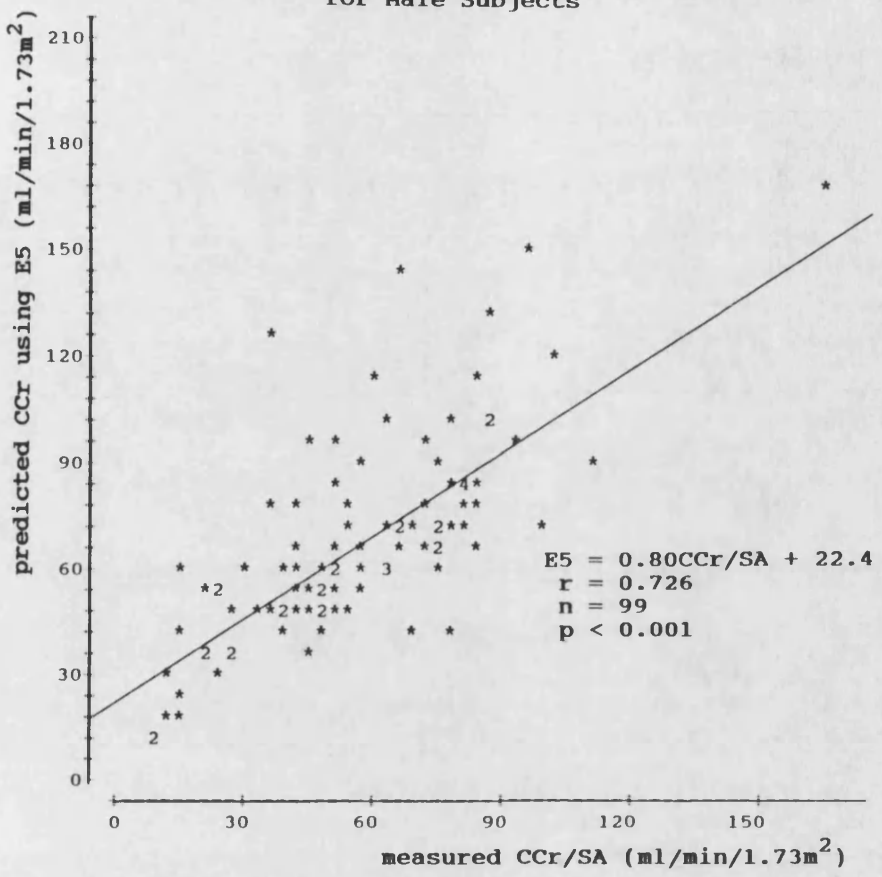


Fig. 3.4.9 Predicted v Measured CCr using E6 for Male Subjects

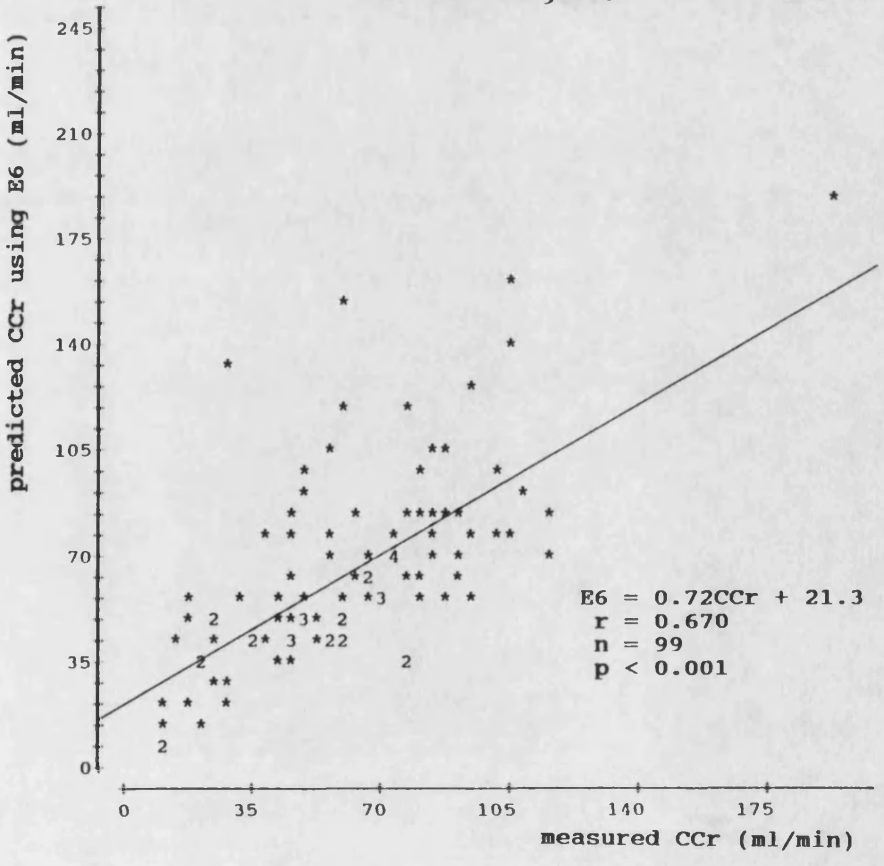


Fig. 3.4.10 Predicted v Measured CCr using E7 for Male Subjects

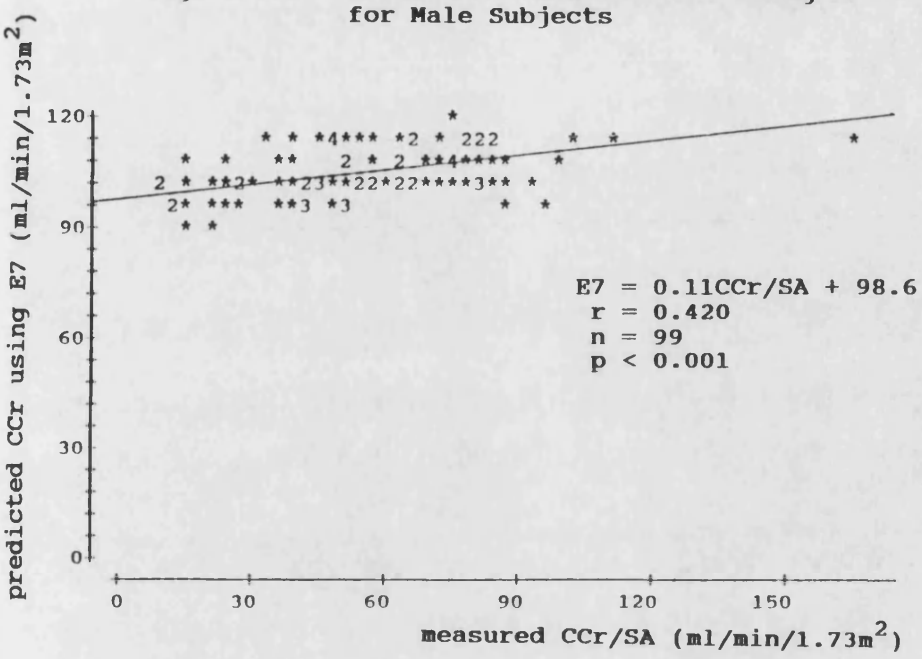


Fig. 3.4.11 Predicted v Measured CCr using E8 for Male Subjects

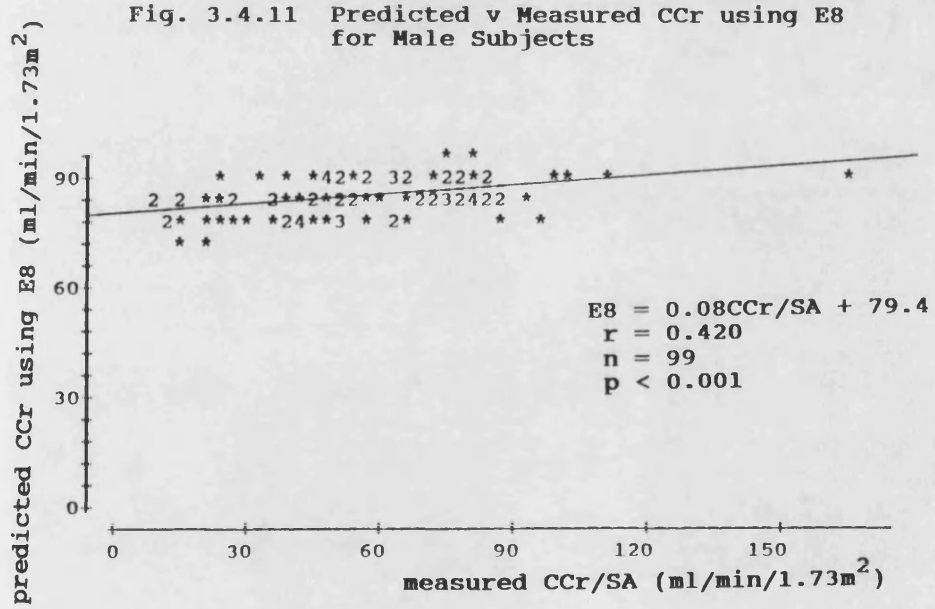


Fig. 3.4.12 Predicted v Measured CCr using E9 for Male Subjects

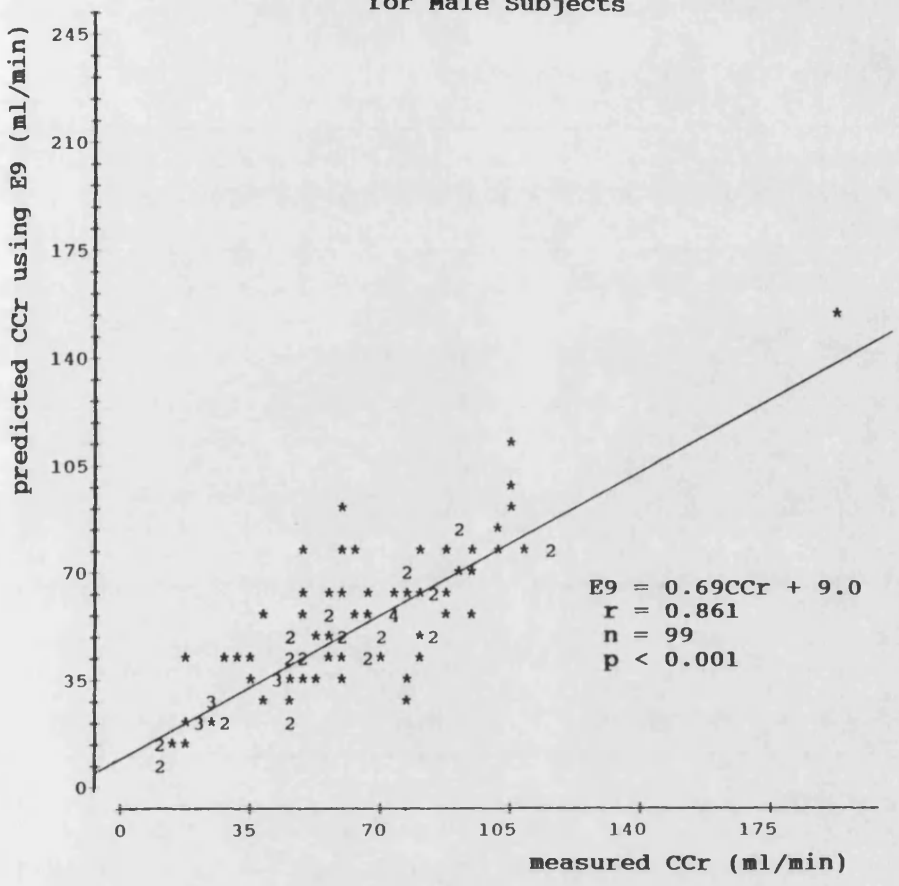


Fig. 3.4.13 Predicted v Measured CCr using E10 for Male Subjects

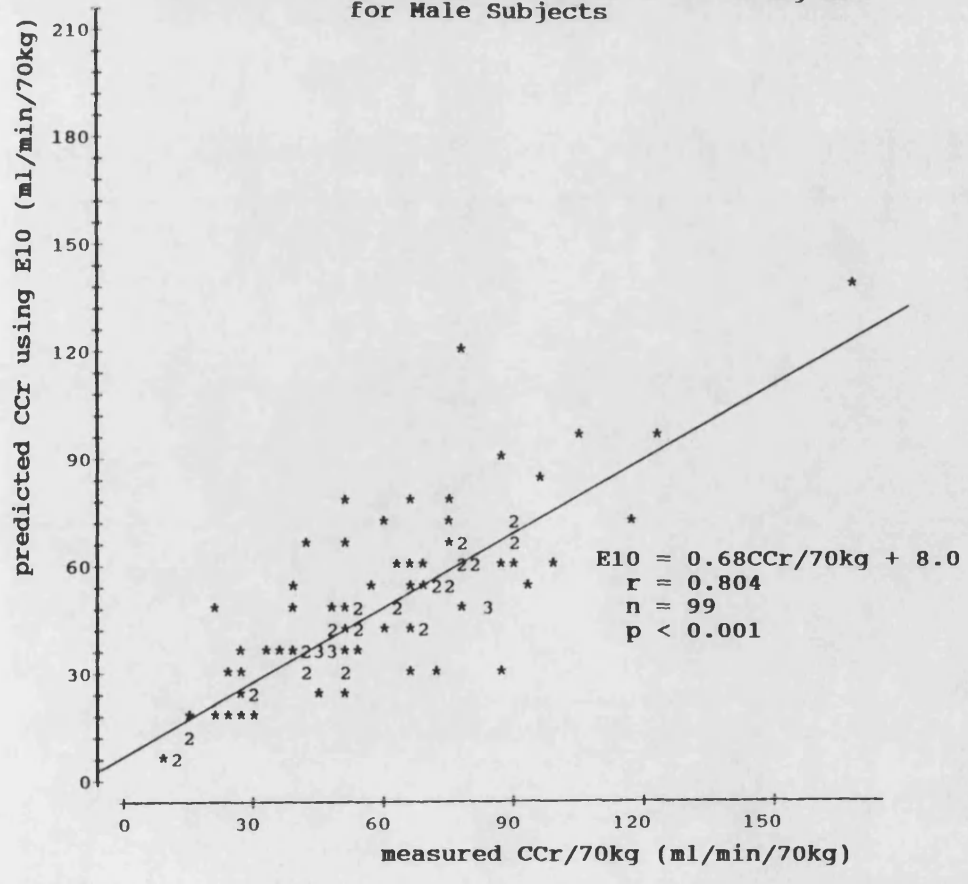


Fig. 3.4.14 Predicted v Measured CCr using E11 for Male Subjects

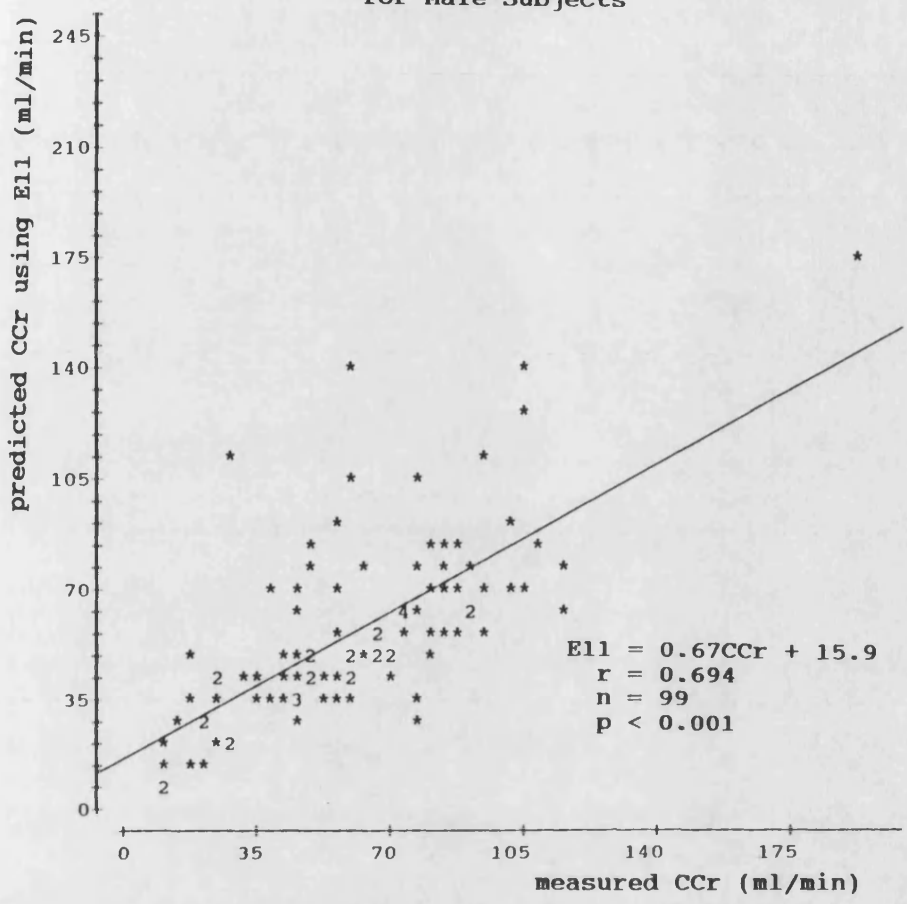


Fig. 3.4.15 Predicted v Measured CCr using E12 for Male Subjects

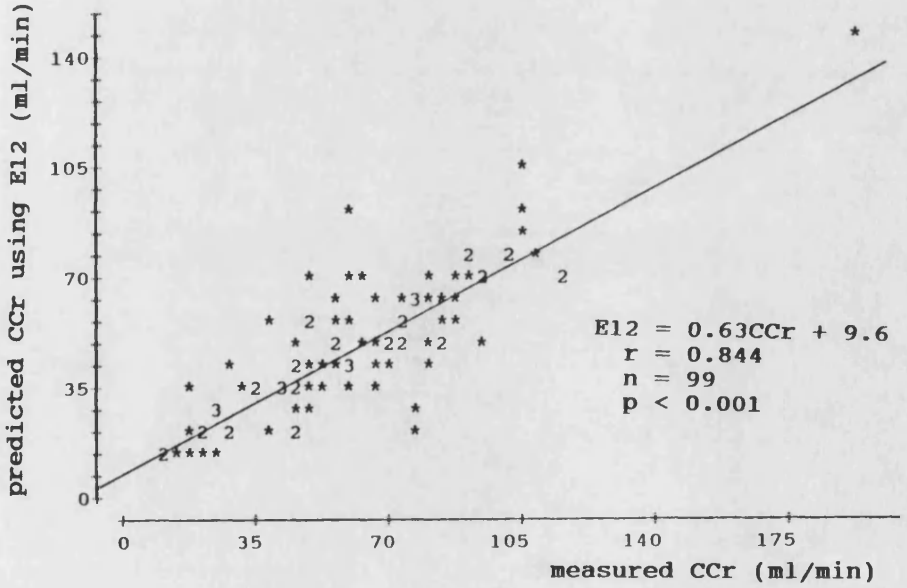


Fig. 3.4.16 Measured 24h CCr v % Predicted CCram for Male Subjects

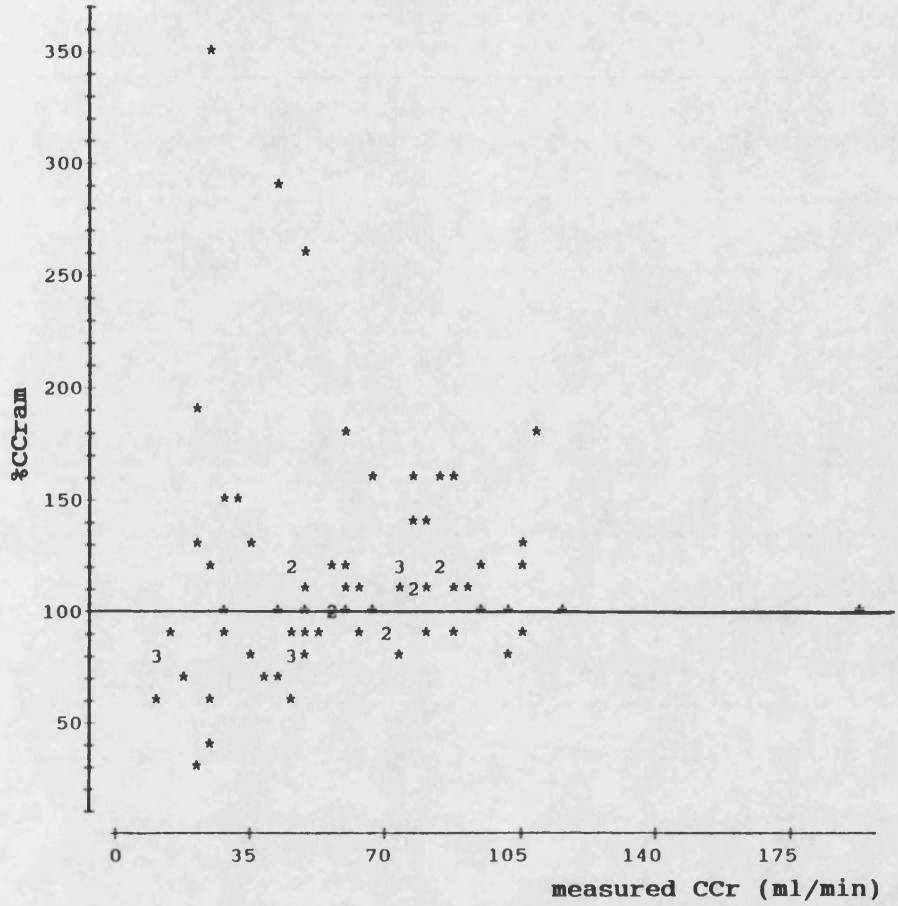


Fig. 3.4.17 Measured 24h CCr v % Predicted CCrpm for Male Subjects

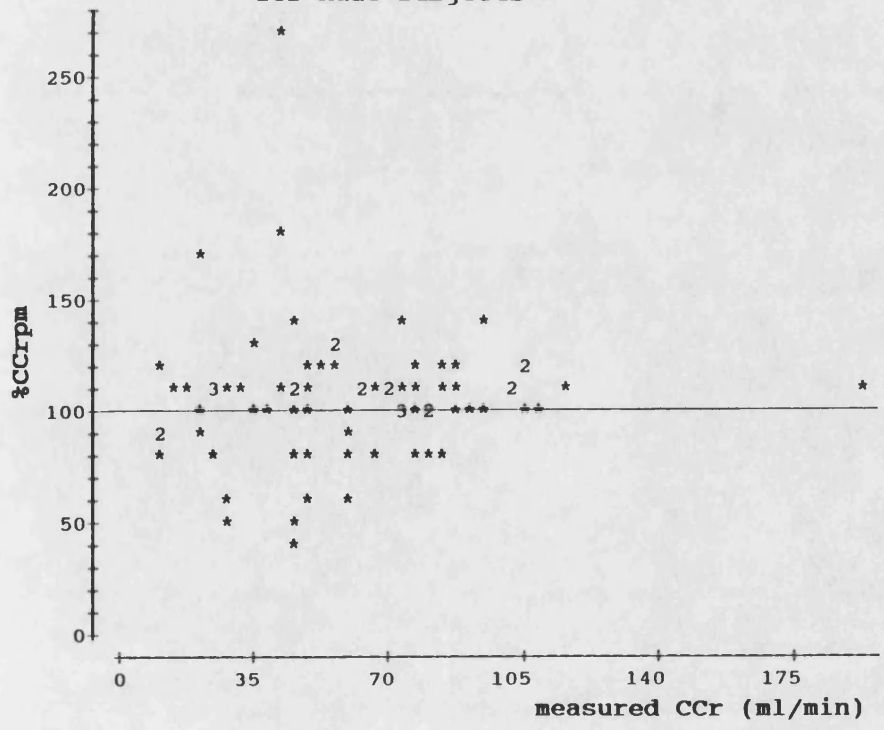


Fig. 3.4.18 Measured 24h CCr v % Predicted CCrn for Male Subjects

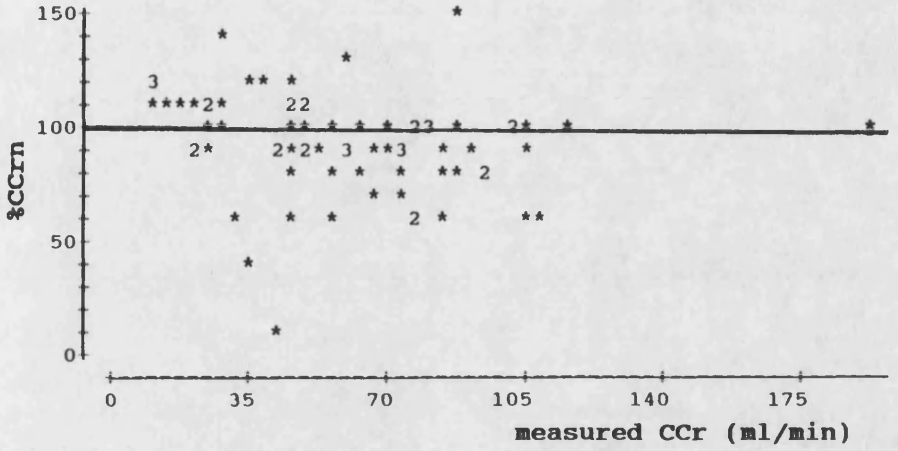


Fig. 3.4.19 Measured 24h CCr v % Predicted CCr Using E1 for Male Subjects

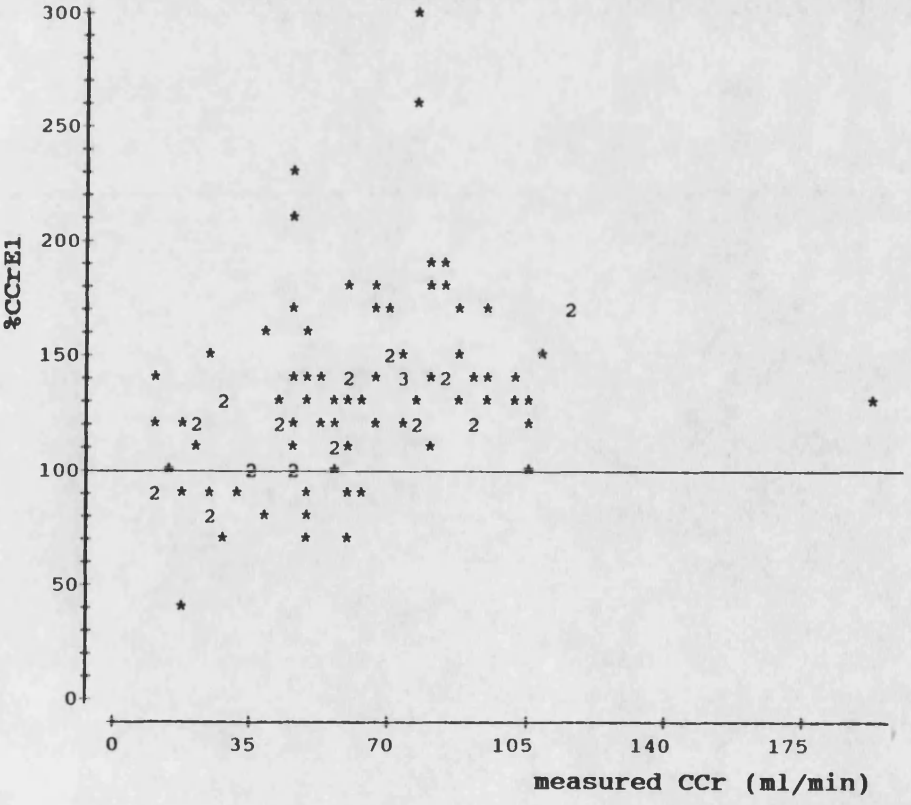


Fig. 3.4.20 Measured 24h CCr v % Predicted CCr Using E4 for Male Subjects

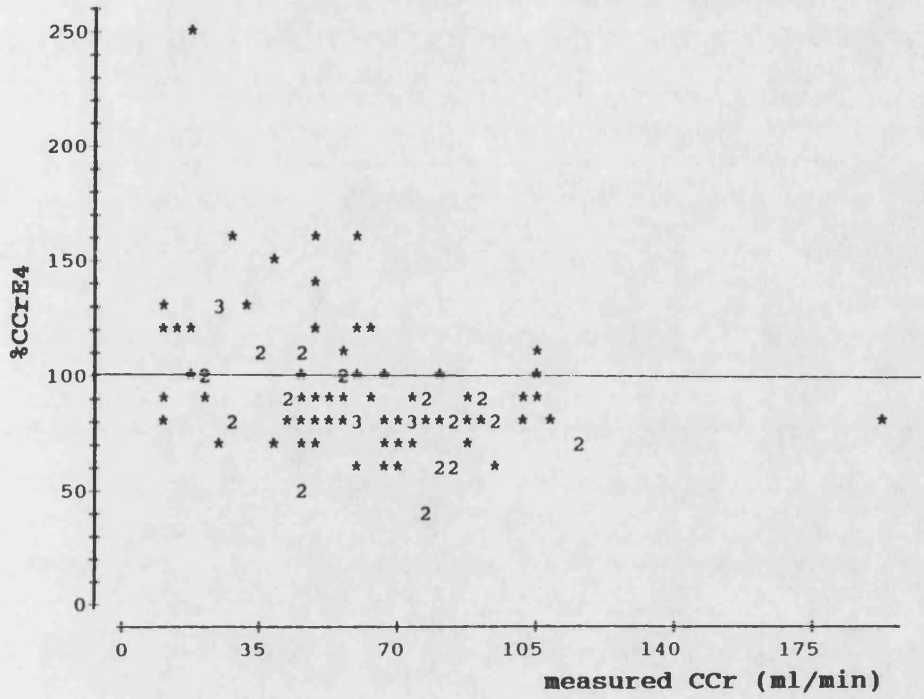


Fig. 3.4.21 Measured 24h CCr v % Predicted CCr Using E9 for Male Subjects

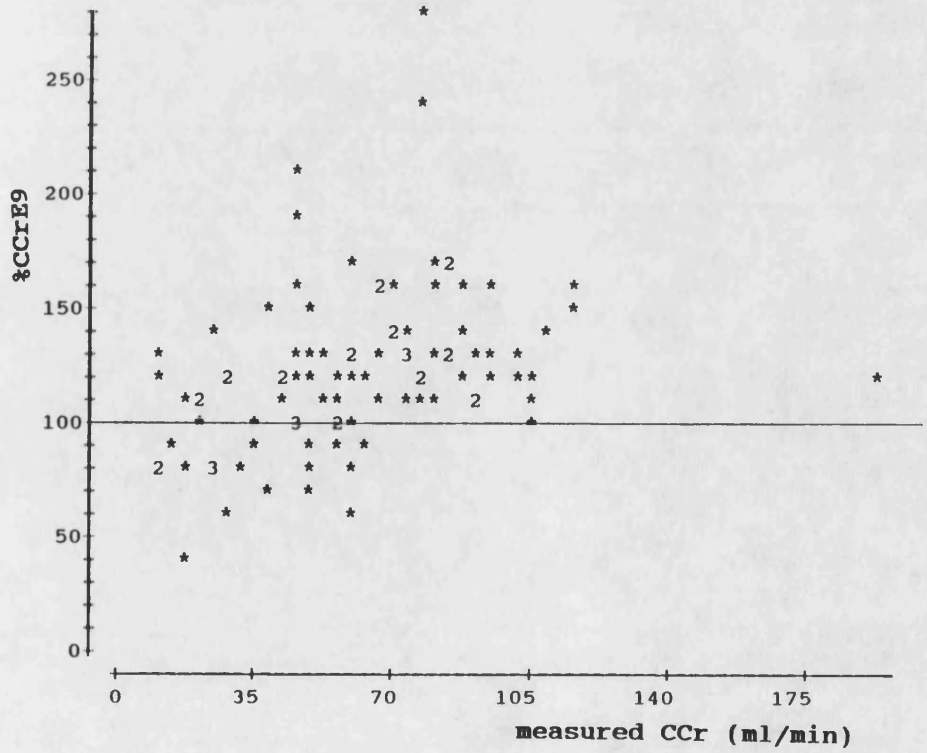


Fig. 3.4.22 Measured 24h CCr v % Predicted CCr Using E10 for Male Subjects

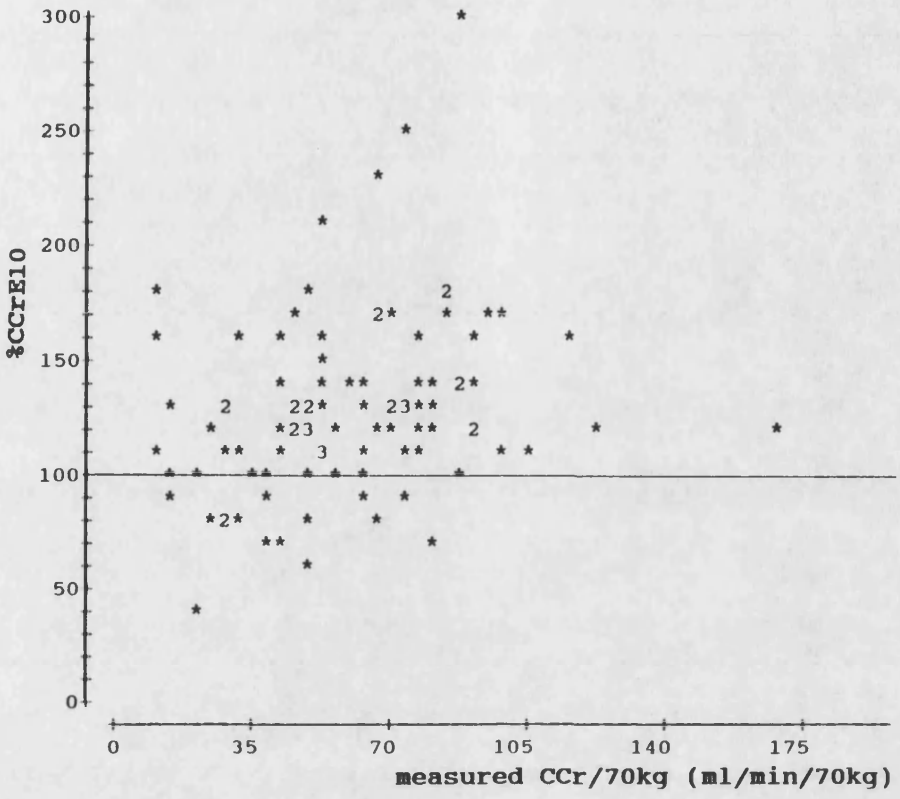
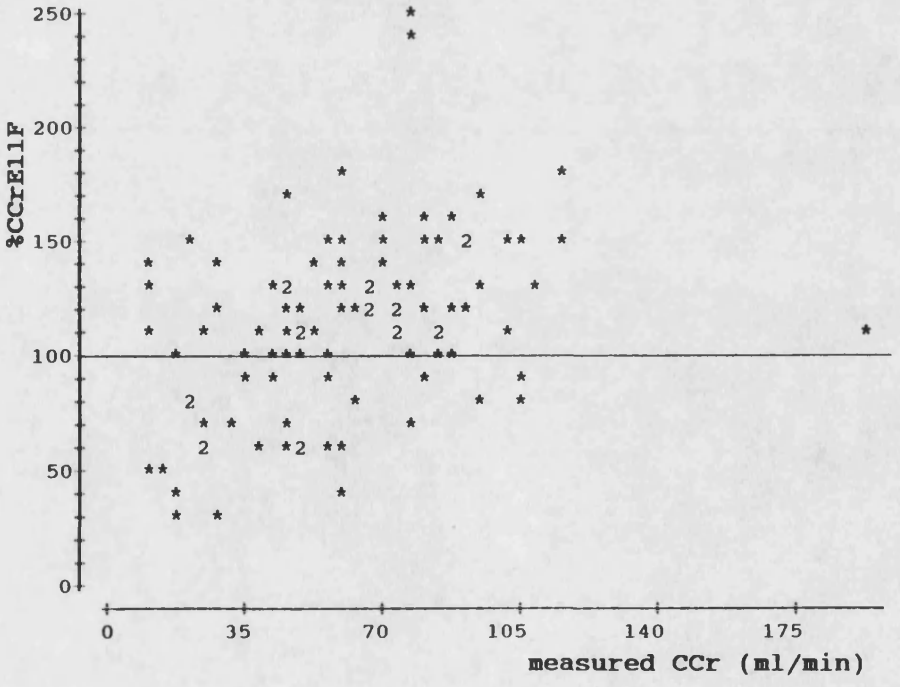


Fig. 3.4.23 Measured 24h CCr v % Predicted CCr Using E11 for Male Subjects



3.5 DISCUSSION

Creatinine clearance was measured and predicted, using 11 equations, one nomogram, and urine collections of less than 24h, in a heterogeneous group of elderly people. The influence of immobility & frailty on 24h CCr and the accuracy of its prediction were investigated.

3.5.1 Creatinine Clearance in Elderly People

Comparison of Male & Female Results

Elderly males studied were matched in terms of age & M.Sc with their female counterparts but they weighed more, had a greater SA, and significantly higher mean values for SCr, UCr & CCr. When CCr was normalized to $1.73m^2$ SA the difference in CCr was no longer significant. This suggests that part of the difference observed may be due to the inequality in weight between the sexes. One longitudinal study of people of working age found UCr excretion to be 33% greater in males, although the difference was reduced when expressed per kg bodyweight. While SCr was also found to be elevated by 21% in the males, CCr was only modestly increased, by about 8% when expressed per kg bodyweight (129). Others found SCr to be significantly higher in healthy males than females even when differences in body surface area were taken into account (130). Another study investigating renal function in 70 & 75 year olds found no sex difference with respect to GFR (131). A fourth study also reported little difference in normalized CCr between elderly males & females (132). Young males usually have a greater proportion of muscle per kg bodyweight than females although this may not necessarily be so in old age when the proportion of muscle to adipose tissue declines (108,132). Creatinine levels in serum & urine are strongly influenced

by total muscle mass (31,129,133,134), and even if the muscle:fat ratio is similar in elderly males & females, males in this study weighed on average 10kg more than females and it is likely that their total muscle mass was greater. This may explain the difference in UCr & SCr observed between sexes.

Equations to predict CCr, often in ml/min, utilize SCr & weight, both of which were significantly different between sexes. Moreover, some include a "female correction factor" to take into account the difference in muscle mass between sexes. One aim of this study was to determine if CCr was more accurately predicted for females in the presence of this factor, and so was more appropriate to analyse male & female data separately and then compare results.

Females

Females studied ranged in age from 60-100y and increasing age was associated with a reduction in mobility. This was accompanied by a reduction in UCr excretion, probably due to the decline in muscle mass associated with a sedentary existence, suggesting that the mobility rating scale gave a reasonable measure of activity. While no correlation was seen between age & SCr, CCr declined with increasing age. This trend has been extensively reported as an inevitable consequence of ageing, occurring even in the absence of active renal disease (123,128,131,135,136). Longitudinal studies have suggested that CCr declines from the age of 30 onwards with an acceleration in rate after age 50 (23,123), although considerable interindividual overlap occurs.

In the present study CCr/SA ranged from 8-167ml/min/1.73m^2 (52±26) with most subjects having a measured CCr below that quoted for a "normal" female of 80-100ml/min/1.73m^2; those

who were immobile and very elderly generally had the lowest values. Other studies have reported similar CCr values for elderly people. Mixed sex groups of elderly people, with mean ages of 75 & 88y, had CCr of 60 & 47ml/min (136,137), while CCr was found to be 57±18 & 77±23 ml/min in groups of females with mean ages of 76 & 83y (132). 19 elderly infirm females (79±6y), had a mean CCr of 51±16ml/min but this value was calculated from two 8h urine collections and all subjects had chronic indwelling urethral catheters (87). SCr was mainly within normal limits despite some subjects having a lower than normal CCr. This will be discussed in more detail later in the chapter. UCr varied greatly within the female group from 240-2441mg (823±313). While some data at the lower end of the range could have resulted from incomplete urine collections, steps were taken to ensure that these were discarded. Other studies have reported UCr excretion to be 1173mg/24h/1.73m^2 & 785mg/24h in groups with mean ages of 67 & 75y (26,136) & 746mg/24h in patients (63±16y) with rheumatoid arthritis (138).

Males

Age of the male group ranged from 60-97y and increased age was again associated with reduced mobility; both old age & immobility were associated with a decline in UCr. As for the females, increasing age was associated with reduced renal function as measured by CCr, which ranged from 10-164 ml/min/1.73m^2 (56±26). Other studies found CCr to be 110 & 97ml/min/1.73m^2 in healthy males between 65-74 & 75-84y respectively (54) which is higher than the present results. UCr varied considerably between males from 302-2624mg/24h (1192±433), and these values are in accordance with others reported. Groups of fit elderly males aged between 65-74y

& 75-84y excreted 1409±25mg & 1259±45mg of UCr in 24h (123)

3.5.2 Influence of Diuretics on CCr

The elderly are known to take a disproportionate quantity of drugs, and diuretics are one of the most commonly prescribed classes. The 36 females & 60 males taking diuretics during the study were compared with subjects who were not, to determine whether diuretic use was associated with a change in CCr. For both sexes a small reduction in CCr was seen for those taking diuretics but this was not significant. Those taking diuretics were older and significantly less mobile which may explain the slight trend in CCr observed. Overall, diuretics do not appear to influence CCr and it was therefore not necessary to treat those taking diuretics separately.

3.5.3 Influence of Frailty on CCr

The elderly are not a homogeneous group but possess a range of physical abilities. The least able are usually found in care differing from their fit counterparts in the community by their limited mobility and altered body composition & posture. It is possible that these factors associated with infirmity may influence renal blood flow and hence renal function. In this study females & males were divided up into groups of fit & frail subjects and compared. For both sexes the frail group was significantly older than the fit group and the way in which frailty contributed to CCr could not be determined. Very fit, fit & frail subjects were therefore age matched & compared in an attempt to eliminate the effect of increasing age on CCr.

For both sexes UCr, SCr, CCr & CCr/SA was not statistically different between very fit & fit groups, suggesting that

chronic but well controlled non-renal conditions are not generally associated with loss of renal function. Subsequent studies in this thesis have compared drug excretion in fit & frail groups and similar results would probably have been obtained if very fit instead of fit subjects had been used. When either very fit or fit groups were compared with their frail age-matched counterparts, a similar trend was seen for both sexes. The frail group was significantly less mobile than the fit since information on mobility was used to categorize subjects. UCr was significantly lower in the frail groups, probably due to a reduced muscle mass resulting from severely restricted mobility. Urine volume was also reduced in frailty although this was only significant for the females. It is possible that the frail group more often had incomplete urine collections, although most frail subjects were in care and urine collections were therefore supervised. Elderly subjects in general are at risk of dehydration and those in care particularly so (24,139), and this may explain the reduced urinary output in the frail group, likely to be a real observation.

CCr & CCr/SA were significantly lower in the frail groups, but no statistical difference was seen for SCr between groups although frailty appeared to be associated with an increased SCr. A SCr value is dependent on both the rate of production & excretion of creatinine, and weight tended to be reduced in the frail groups. In addition, frail subjects are more likely to have muscle atrophy as a result of restricted mobility & chronic illness, and UCr was seen to be significantly reduced in this group. Thus the reduction in CCr associated with frailty would not necessarily be

reflected in a raised SCr. Another group of sick elderly people, mean age 80y, had a GFR of 41ml/min when measured by the single shot ^{51}Cr-EDTA method. Although the authors concluded that GFR was reduced in sick elderly people compared to their fit counterparts no parallel trial was carried out in the fit elderly, and results were compared with those reported by other groups who had measured GFR by other means (135).

3.6 PREDICTION OF CREATININE CLEARANCE

In clinical practice GFR may be estimated by measuring endogenous creatinine clearance. While the accuracy with which CCr predicts GFR is subject to debate CCr measurement nevertheless remains in clinical usage as it is relatively easy and inexpensive to carry out compared to other methods available such as ^{51}Cr-EDTA, ^{99m}Tc-DTPA or inulin clearance (31,33,36,134). To determine CCr a timed 24h urine collection is required together with a single blood sample. Concentration of creatinine in urine & serum are then measured, and since the volume of urine passed in 24h is known, a clearance value can be calculated for creatinine. One of the main criticisms of this method of GFR estimation is that an accurately timed 24h urine collection is at best difficult to achieve and at worst impossible in, for example, a confused, incontinent elderly patient. Besides this difficulty there is the obvious delay in obtaining a result, a particular drawback when GFR needs to be known before certain drugs can be prescribed. Since analyses of both urine & serum are required the cost of the test is increased and additional errors are introduced, in addition to those inherent in timing & collecting urine (140).

Within-subject variation in UCr excretion may be wide even in healthy people where the coefficient of variation has been found to be around 13%, even when urine is collected under strictly controlled conditions (141,142). Such variation is therefore likely to be due to true day-to-day alterations in creatinine production & excretion rather than methodological inaccuracies.

Studies have shown that not all creatinine is excreted via glomerular filtration, with some eliminated via tubular secretion; this route is thought to become more important in chronic renal failure, although proteinuria does not appear to exert a significant effect on the degree of secretion (123,143,144,145,146,147). Thus CCr may over-estimate GFR, although a good correlation has been observed between CCr & GFR as measured by inulin clearance where some of the likely causes of variation (eg diet & activity) do not exist (148).

It has been suggested that more information is gained from knowledge of SCr alone (34,35). This has the advantage of speed & convenience, with a reduction in cost. However, SCr is usually assayed using a modification of the Jaffe reaction which has been shown to be non-specific for creatinine. Other substances able to react with the active moiety include protein, glucose, ascorbic acid, keto-acids, pyruvate, acetone, bilirubin & cepha antibiotics (35,149, 150). "Total chromagen" (creatinine + non-creatinine chromagens) rather than creatinine alone is measured, and this is an obvious source of inaccuracy when a SCr value is relied upon. In the present study SCr & UCr were measured using HPLC in order to eliminate this source of analytical error, since this method has been shown to be more specific

(115,151,152,153).

A postprandial rise in SCr & UCr has been reported after a cooked meat-containing meal, which can give a falsely elevated SCr level, although CCr is not influenced by dietary factors (153,154,156). In this study blood was taken in the morning following the 24h urine collection in all subjects, following an overnight fast or a meat-free breakfast. Most subjects were asked about their meat intake but few consumed quantities near to the 225g or more of lean meat used in other studies.

A further criticism of the use of SCr alone to estimate renal function is that the normal range quoted is much wider than the variation in SCr observed for an individual, even when analytical errors are taken into account (157,158 159). Since SCr is related to GFR in a reciprocal fashion, when renal function is normal or only mildly impaired small changes in SCr represent large changes in GFR (35,36,134). This can be a particular problem in the frail elderly and in specific conditions such as rheumatic disease, paralysis and burns where the disease process and physical inactivity leads to a reduced muscle mass and lowered creatinine production (31,123,131,136,138,160,161,162,163). Abnormal body compositions may therefore produce a normal SCr in the presence of renal impairment and an over-estimation of CCr when the classical equations are employed.

One solution to this problem would be to develop more reference ranges for SCr for a variety of body weights & ages (35,134,158,163). A reduction in urine collection time would offer an advantage over the traditional 24h urine collection, particularly for those groups of patients where SCr is difficult to interpret (36). Alternatively, a better

method for predicting CCr from SCr would enable more easily interpreted results to be obtained from SCr without the inherent difficulties associated with a urine collection.

3.6.1 Prediction of CCr From Reduced Urine Collections

Few studies have evaluated the accuracy of using a reduced urine collection interval to estimate 24h CCr despite the fact that the difficulties inherent in carrying out a 24h urine collection are well known. Studies investigating the change in urine output with time of day in healthy young subjects have found a reduction overnight (26,164). However this pattern is reversed in elderly subjects who excrete a higher proportion of water, sodium, potassium & solute output at night irrespective of fluid intake during the evening (26,165). Frail elderly females resident in hospital were nursed supine in bed for 3 days or upright in chairs for an 8h period each day for 3 days and their urine output examined (166). All exhibited a complete absence of "morning tide" of water & electrolytes regardless of position. The first day of total bedrest was associated with a large diuresis, and patients nursed in chairs also produced a large nocturnal urine output when returned to bed. After a 3 day period of bedrest the first session of nursing in chairs was characterized by oliguria. The authors concluded that changes in posture were important determinants of urinary output patterns, and may account for the frequent nocturia seen in elderly subjects. Another study investigated urine output in healthy males during normal activity & rest in the antiorthostatic position (167). While diuresis was enhanced in the antiorthostatic position, SCr & CCr did not appear to change with position. Conversely, two other studies found

an increase in both diuresis & CCr in healthy males & patients with fluid-retaining states in the supine position compared to when sitting upright (168,169).
Those who have measured the circadian variation in CCr or GFR have found a small but pronounced reduction in CCr occurring during the night in healthy young subjects (156, 164,170). This pattern remains when subjects are fasted to eliminate any dietary effect, and is also seen in paralysed patients and is therefore unlikely to be due to changes in muscle activity. CCr has been observed to change little during a 2h period of normal activity compared to that during 2h of immobilization in healthy males (171) although a period of unusually heavy exercise may give rise to an increased UCr & SCr (134,142). However, strict control of diet & activity is reported to have little influence on the within subject variation in UCr (142).

In this study urine was collected in aliquots in 76 males & 116 females to determine whether urine collections of less than 24h could be used to accurately predict 24h CCr.
Urine was collected over time periods of 4-12h to represent morning, afternoon/evening and night urine collections. Since urine was passed as usual collection periods varied in length although the night collection always represented that urine passed during the hours spent in bed overnight.
For both male & female groups CCram, CCrpm & CCrn, when plotted against 24h CCr had a high correlation coefficient, a gradient near to unity and a y intercept close to 0 (appendix C7). CCram & CCrpm overestimated CCr by a mean figure of between 2-8 ml/min while CCrn underestimated CCr by about 5-10ml/min. The small reduction in CCr observed overnight is in agreement with other studies following the

diurnal variation in CCr. Other comparisons of CCr from urine collections less than 24h with traditional 24h CCr have generally chosen shorter time periods over which to collect urine. One study compared CCr from a 1h collection with a 24h collection in healthy young males and found good correlations between them (172). A second compared CCr calculated from urine collections of 2, 4 & 24h with GFR measured using ^{99m}Tc-DTPA (147). GFR was overestimated by all urine collection methods with the 2h collection seen to be least accurate. Patients with CCr < 50ml/min were more likely to have CCr overestimated than those with CCr > 50 ml/min. No similar studies have been carried out in elderly people. A very short urine collection would not be practical in the elderly who more frequently suffer from incomplete bladder emptying, difficulty in micturition and dehydration. The accurate timing required would also pose problems on a busy ward. Although water loading to increase urine flow was used to facilitate a one hour urine collection, results were less accurate (172), and problems would be experienced in the elderly. One study saw CCr increased in healthy young & elderly males following water loading, with the elderly group appearing less able to efficiently excrete excess water (27).

The accuracy of CCr prediction using urine collection periods less than 24h was compared between those with CCr > 50ml/min & those with CCr < 50ml/min. For both females & males the correlation between measured & predicted CCr remained good and was not compromised at the extremes of 24h CCr. Diuretic use did not appear to influence accuracy of CCr prediction for any of the collection periods. Males & females were divided up into groups of very fit,

fit & frail age matched subjects and CCr prediction compared. While frailty was strongly associated with a reduction in 24h CCr, predictability was not generally compromised in any group. For each group CCrn remained slightly less than 24h CCr while CCram and less so CCrpm were slightly greater than 24h CCr.

3.6.2 Prediction of CCr from SCr and Equations

From as far back as 1959 groups of workers have attempted to devise either an equation or nomogram to accurately predict CCr from SCr. Early equations consisted of a simple mathematical relationship between SCr & CCr but their validity was found to be limited. The reduction in CCr with increasing age and the relationship of SCr to bodyweight or lean body mass led to the development of more complex equations and to date 12 equations & 4 nomograms exist for use in adults (31,118,119,120,121,123,125,126,128 133,135,173,174,175,176,177,178). A further variety of studies have been carried out to evaluate the use of these formulae in healthy young & elderly subjects and in patients with a variety of conditions.

In the present study the accuracy of CCr prediction using a variety of formulae was assessed in a heterogeneous group of elderly people. A 24h urine collection was successfully completed by 146 females & 99 males aged between 60-100y, and used to calculate CCr. Each individuals SCr was then used to predict CCr using 11 equations (E1-E11) and 1 nomogram (E12), and the absolute & percent differences between measured & predicted CCr were determined.

When predicted v measured CCr was plotted for each equation and both sexes a large variation in accuracy between equations was seen; those which relied upon a simple

relationship between SCr & CCr without the incorporation of age or weight factors were the least accurate. Although these equations are the most easy to use and remember, they have little place in the clinical practice due to their poor predictability, and so they were not used any further in the statistical analyses. Those equations found to predict CCr best were similar for both sexes and were E1 (Cockcroft & Gault), E4 (Jelliffe & Jelliffe), E9 (Mawer), E10 (Hull) & E11 (Gates).

In only one case (E11) did the "female correction factor" enhance predictability for females and the other selected equations were used in the same form for both sexes. While few studies have investigated the usefulness of the female correction factor, those studies that have are divided in opinion. Two studies have found the use of a correction factor to be unnecessary in elderly females (137,179), although in the former study the sample size was small. Others studies found a correction factor of 0.9 & 0.84 more appropriate when E1 & E3 were used (180,181). Body composition is known to change in old age, with the proportion of fat to muscle increasing and body water decreasing (108,182). Thus in the elderly it is possible that males & females differ little in respect to muscle:fat proportions particularly when muscle atrophy is present. Accuracy of CCr prediction was similar for the 5 best fit equations for both sexes. Correlation coefficients for predicted v measured CCr ranged from 0.716-0.858 & 0.694-0.862, gradients from 0.70-0.78 & 0.64-0.71, and intercepts from 11.6-13.1 & 8.0-15.9 for females & males respectively. Mean predicted CCr agreed well with mean measured CCr for each equation, but the accuracy of prediction was not

constant across the spectrum of measured CCr. When predicted v measured CCr was plotted those with the lowest measured CCr tended towards overprediction, and those with the highest CCr tended towards underprediction. This pattern was most pronounced for the females.

Out of the 5 best fit equations E10 & E11 were the least accurate and most variable for both sexes. The most accurate and least variable equations were E1 & E4 for the females and E4 & E9 for the males.

Using the 5 "best fit" equations subjects were divided into groups of those with a CCr > 50ml/min & those with a CCr < 50 ml/min. For both sexes the error of prediction was seen to alter between groups. Those with measured CCr > 50ml/min tended to have CCr underestimated by the equations, while those with a measured CCr < 50ml/min tended to have CCr overestimated, and the change in prediction accuracy between the two groups was significant in all cases.

The line of measured v predicted CCr for the females was shifted to the left of that for males with the degree of overprediction greater and underprediction lower for the females. The use of a female correction factor would have improved the position of the female line with respect to the male line, although the correlation coefficient would not be improved. It seems likely that a correction factor of 0.95 would be more appropriate, if thought necessary, in this group, since 0.90 or 0.85 reduced the accuracy of prediction considerably.

Using a similar technique the accuracy of CCr prediction for females & males taking diuretics was compared to those who did not and appeared to be independent of diuretic use.

The accuracy of CCr prediction in very fit, fit & frail age

matched groups of males & females were then compared. For the females, very fit & fit groups had comparable measured CCr and while CCr tended to be underpredicted the degree of underprediction was similar between groups. The very fit males tended to have a greater measured CCr than the fit group, and consequently the degree of underprediction was significantly greater in the very fit group. Frailty was associated with a significantly reduced measured CCr for both sexes when compared with their age matched fitter counterparts. For the females, the frail group had the lowest measured CCr & exhibited consistently overpredicted CCr using the selected equations. For the males, very fit, fit & frail groups all tended to have CCr underpredicted, although the degree of underprediction was lowest in the frail group who had the lowest measured CCr.

Other studies have compared the accuracy of CCr prediction using a variety of equations on selected groups of subjects. Most have investigated CCr prediction in healthy people with a range of ages and found a good correlation between measured & predicted CCr (118,119,128,174,176,177, 180,181,183,184,185). E1 predicted GFR measured by ^{99m}Tc-DTPA or ^{51}Cr-EDTA at least as accurately as 24h CCr in two studies although only 35 patients carried out 24h urine collections in the former study (147,185). Two groups investigated CCr prediction in elderly people (88 & 70y) and found good correlations between measured & predicted CCr using E1 (132,137). Another group found E12 to best predict CCr for elderly people (72y) with measured CCr of between 25-89 ml/min (186). However, debilitated elderly females resident in nursing homes were found to have CCr overpredicted using E1 & E3, although urine collections of

8h were used to calculate a 24h CCr without validation (87). These subjects were also chronically catheterised, which may have adverse effects on renal function.
Several studies have examined CCr prediction in patients with renal impairment and found differing results, although in all cases renal function was stable; only E4 & E9 may be used when renal function is changing. CCr was predicted accurately in a group of Chinese patients with renal impairment using E1 (177). Another study found CCr predicted more accurately using E9 & E10 than E1 & E3 in patients with renal dysfunction (126). A further study concluded that E2 best predicted CCr if patients had a SCr > 1.5mg/dl, while CCr of those with SCr < 1.5mg/dl was best predicted using E2 (178).
Accuracy of CCr prediction appears to breakdown for patients with hepatic dysfunction using E1,E3,E9 & E10 (126). Others have found CCr poorly predicted in gross RVF and low cardiac output states using E12 and CCF using E1,E3 & E9 (187). Males with extensive burns have been found to have CCr overpredicted using E2 & E3, with E1 & E12 giving better results although the tendency towards overprediction remained when measured CCr < 60ml/min (188). This study also found that the use of lean body mass in place of actual bodyweight did not improve accuracy of prediction. CCr overprediction has also been found for both paraplegics & tetraplegics using E1, with the authors recommending a correction factor of 0.8 for paraplegics & 0.6 for tetraplegics (160). Patients with rheumatoid arthritis also had CCr overpredicted by 20ml/min using E1 & E12 (138).

3.7 CONCLUSIONS

CCr was measured & predicted for a range of elderly people, and those who were frail had a reduced renal function when compared to their fit counterparts if age-sex matched.

Urine collections of less than 24h predicted CCr better than any equation or nomogram studied, for both males & females. Predictability of CCr from a reduced urine collection was not influenced by the magnitude of measured CCr, or compromised in frailty. Those taking diuretics had CCr predicted equally well as those who were not. CCrpm & CCrn were the most accurate and least variable, with CCrpm tending to overpredict CCr slightly and CCrn tending to underpredict CCr.

Given that CCr tends to overpredict GFR, it would seem most appropriate to employ an overnight urine collection to estimate CCr and GFR. This could simply be carried out if urine passed before going to bed was discarded, and the time noted. All urine passed overnight would then be collected, up until and including that passed on getting up, when the time would again be noted. Blood for SCr determination could then be taken, and the two specimens sent together for analysis.

Of the 11 equations & 1 nomogram studied none predicted CCr as accurately as reduced urine collections. The variability was greater, and the predictability varied with magnitude of measured CCr. Those with the lowest measured CCr tended to have CCr overpredicted by the greatest degree. Frailty is associated with a reduced CCr and it is on this population that the formulae are most often applied, and who are at greatest risk of adverse drug reactions.

The literature suggests that the greatest inaccuracies in CCr prediction occur in subjects suffering from conditions which produce an abnormal body composition. Therefore weight loss & muscle atrophy commonly seen with frailty would be likely to cause inaccuracies in CCr prediction, and results from the present study confirm this.

Data from this study suggests that use of urine collections less than 24h, particularly when carried out overnight, predicts CCr more accurately than any of the existing equations or nomograms. The accuracy of CCr prediction using a reduced urine collection is valid for both fit & frail elderly people, even in the presence of renal impairment, and immobility and diuretic use do not appear to affect the results.

CHAPTER FOUR

FRUSEMIDE EXCRETION IN ELDERLY PEOPLE

4.1 INTRODUCTION

It has been estimated that one in three elderly people regularly take diuretics. Currently one of the most widely prescribed diuretic formulation is co-amilofruse (frusemide 40mg + amiloride 5mg; 39). Frusemide excretion in man has been the subject of a number of studies which have used both healthy volunteers and patients with particular diagnoses. However, there appears to have been little work carried out specifically in elderly patients with multiple pathology with associated immobility and data compared with those of similar age whose lifestyle is not restricted by chronic disease. In this study frusemide (FRU) excretion was studied in elderly people taking FRU chronically, who were either "fit" (well controlled chronic illness, mobile & living independently) or "frail" (similar diagnoses but immobile & lack of independence) in an attempt to determine the influence of immobility and frailty on FRU elimination.

4.2 PROCEDURE

Fit & frail elderly people aged over 64y were invited to take part in the study. All were non-acutely ill and had been regularly taking FRU as frusemide (FS) or Frumil (FM) tablets, for chronic illness of cardiovascular origin, for a minimum of one month. Exclusion criteria are given in 2.2. Each subject was provided with an information sheet (appendix D1, standard or enlarged type) and given the opportunity to question Dr L Parker (GP trainee) or myself about the study prior to its commencement.

On the morning of the study subjects were asked to empty their bladder and then take their usual FRU tablet with a glass of water, in the upright position. FRU dose varied according to individual prescriptions which were not altered for the study. Tablets were taken after a light breakfast of toast or cereal then food & drink was avoided for the next hour, after which time both were taken freely.

During the study subjects were questioned about their day-to-day activities & mobility. They were categorized as fit or frail and had their mobility scored according to the mobility rating devised for the study (Appendix A1, A2). The form given in appendix D2 was completed during the study when co-medication was noted and subjects were weighed & measured. Demographic details & drugs taken are given in Appendix D3.

5-10ml of blood was taken at 2,4,8 & 24h & urine collected for 24h after dosing. FRU is light sensitive so all samples were collected and stored in lightproof containers. FRU was measured in all samples, UCr in all urine samples, and SCr in serum at 24h. Urine samples collected over 24h were assayed in 3 aliquots each of about 8h, with the final aliquot spanning the time spent in bed overnight. Methods of analysis for creatinine & FRU are given in 2.3.4 & 2.3.5

Elimination $t_{1/2}$, AUC, apparent serum clearance (Cls), Cls/kg, renal clearance (Clr), Clr/kg, Clr from 0-6h (Clr 0-6), and CCr were calculated as per 2.4.
Correlations were determined using Spearmans correlation with significance levels taken as $p<0.01$ to reduce the chance of Type II error. Groups were compared using the Mann-Whitney test when significance levels were $p<0.05$.

4.3 RESULTS OF PHARMACOKINETIC PARAMETERS

35 subject (22F) successfully completed the study; results from individuals for FRU & creatinine measurements are given in appendices D4 & D5, and summarized in appendix D6.

4.3.1 Frumil v Frusemide

Data from FM & FS groups were compared using the Mann-Whitney test with results given in appendix D7. A significant difference was seen for elimination $t_{1/2}$ which ranged from 0.82-11.90h (4.49±2.85) for FM & 1.36-20.17h (9.83±5.55) for FS ($p<0.01$). Clearances were not significantly different between groups although the FS group consistently had a greater variability & s.d. No other statistical differences were seen. However, the above differences in kinetic parameters were considered important enough for the FS & FM groups to be treated separately.

4.3.2 Females v Males

Data were divided into male & female groups for both those taking FM & FS and compared using the Mann-Whitney test with results given in appendices D8 & D9.

The FM group comprised of 16 females & 9 males. UCr was significantly greater in the male group (1279±252mg M, 776±258mg F;$p<0.001$) as was SA ($p<0.02$) & weight ($p<0.03$). No other significant differences were seen.

6 females & 4 males took FS. When the sexes were compared no significant differences were seen.

From these results it seemed most appropriate for FM & FS groups to be of mixed sex since no significant differences were seen between sexes in terms of FRU clearance

4.3.2 Subjects Taking Frumil

25 of the 35 participants in the study took either 1 or 2

FM tablets and a summary of results & correlations from this FM group are given in appendices D7 & D8.
Age ranged from 65-100y (80±9), correlating with urine volume ($p<0.001$). Mobility score ranged from 1-4 and correlated with CCr ($p<0.01$).
Elimination $t_{1/2}$ showed a wide interindividual variation of 0.82-11.90h (4.49±2.8) correlating with no other parameter. Apparent serum clearance, Cls, exhibited an 18 fold range from 16-279ml/min (116±69), correlating with Clr ($p<0.001$). Cls/kg showed a narrower interpatient range of 0.31-4.36 ml/min/kg (1.72±0.99). Renal clearance, Clr, was also highly variable with a range of 10-197ml/min (81.8±54.5), correlating with %Du ($p<0.01$); Cls & Cls/kg ($p<0.001$). While Clr/kg was less variable, Clr 0-6h was more so (range 7-520ml/min; 170±131).
Dose of FRU ranged from 40-80mg and did not correlate with any other parameter. Urine volume/24h varied between 472-2761ml (1515±564). Percent dose in urine, %Du, ranged from 8.3-69.1% (36.2±16.2), correlating only with Clr.
UCr ranged from 365-1524mg and correlated with CCr/SA, urine vol & weight ($p<0.01$); CCr & SA ($p<0.001$). SCr varied from 1.00-4.19mg/100ml (1.86±0.84), correlating with CCr.
CCr varied from 9-77ml/min (41.8±19.8) and correlated with M.Sc, SCr & urine volume ($p<0.01$); UCr ($p<0.001$). CCr/SA ranged from 11-67ml/min/1.73m^2.

4.3.4 Subjects taking Frusemide

10 subjects studied took FS at a dose varying from 40 to 120mg. A summary of results & correlations are given in appendices D12 & D13.
For this group age ranged from 67-89y (77±9). Elimination $t_{1/2}$ varied widely from 1.36-20.17h (9.83±5.55). Cls varied

from 14-422ml/min (114±139) & Clr from 11-289ml/min (79±91) Numbers were limited in the FS group, and so while many trends seen were similar to the FM group, few reached significance. Significant correlations similar to the FM group were: Clr v Cls & Cls/kg. Correlations reaching significance in the FS group but not in the FM group were : urine volume v Cls ($p<0.01$); & $t_{1/2}$ v Clr ($p<0.01$).

4.4 COMPARISON OF PARAMETERS FOR FIT V FRAIL GROUPS

Since pharmacokinetic parameters did not appear to be affected by sex, groups studied were of males + females. Proportionally more frail than fit subjects were found to be taking FS. Since $t_{1/2}$ was increased & Cls tended to be reduced in those taking FS, data from subjects taking FS & FM were analysed separately.

4.4.1 Subjects Taking Frumil

Results from fit (n=14) & frail (n=11) subjects taking FM are given in appendix D14 and Figs. 4.4.1 to 4.4.6.
The frail group were on average 8y older than the fit group who were significantly more mobile. FRU dose differed between groups - all fit & 6 frail subjects took 40mg FRU, 5 frail subjects took 80mg of FRU.
No significant difference was seen between groups for $t_{1/2}$ (4.00±3.33h fit, 5.12±2.06h fr), although it tended to be lower in the fit group who exhibited the greater range. CCr & CCr/SA were significantly greater in the fit than frail group (CCr: 51±18ml/min fit, 30±17ml/min fr.;$p<0.02$). Urine volume also tended to be greater in fit subjects although %Du was comparable between fit & frail groups. Cls & Clr were consistently greater in the fit subjects taking FM, even when corrected for bodyweight, but this was

not statistically significant.
Fit & frail groups were similar for UCr, SCr, weight & SA.

4.4.2 Subjects taking Frusemide

Results from fit (n=3) & frail (n=7) subjects taking FS are given in appendix D15. Both groups were similar in age (72±6y fit, 80±9y fr) but fit subjects were significantly more mobile. All fit & 4 frail subjects took 40mg FRU, 2 frail subjects took 80mg & 1 took 120mg.
The fit group taking FS tended to have a lower $t_{1/2}$ but this was not significant (4.00±3.33h fit, 5.12±2.06h fr). Cls & Cls/kg were again reduced in the frail group as were Clr, Clr/kg & Clr 0-6h, but none of these observations reached statistical significance. Groups were similar for urine volume, %Du, UCr, SCr, CCr, weight & SA.

4.4.3 Age-Matched Group taking Frumil

The inequality in age between fit & frail groups for those taking FM meant that the effects of frailty & age could not be separated. It was not possible to age-sex match subjects and so sub-groups were formed of the 8 oldest fit & 7 youngest frail subjects. Results are given in appendix D16. Despite the equalization in age between groups, fit subjects remained significantly more mobile than frail subjects. Elimination $t_{1/2}$ was found to be significantly greater in the frail group (3.69±3.53h fit, 5.09±1.68h fr.; $p<0.05$). However, while clearances again tended to be reduced in the frail group significance was not reached. No other statistical differences were seen.

Fig. 4.4.1 Distribution of FRU t1/2 in Fit & Frail Elderly People taking FRUMIL

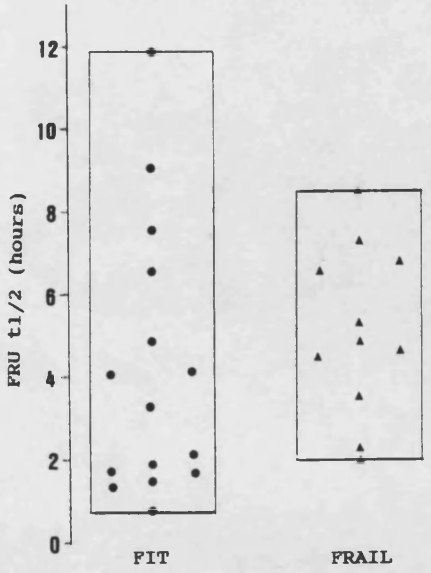


Fig. 4.4.2 Distribution of CCr/SA in Fit & Frail Elderly People taking FRUMIL

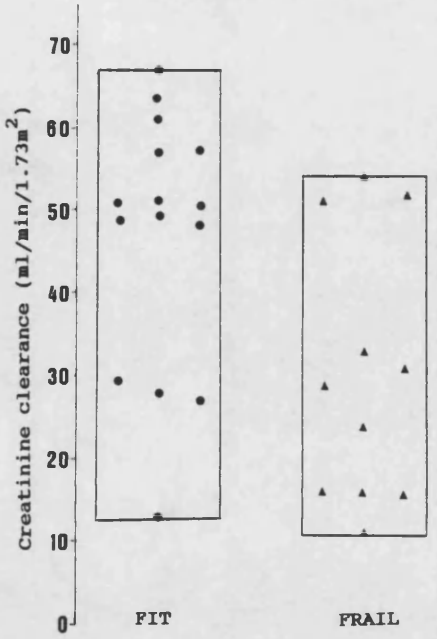
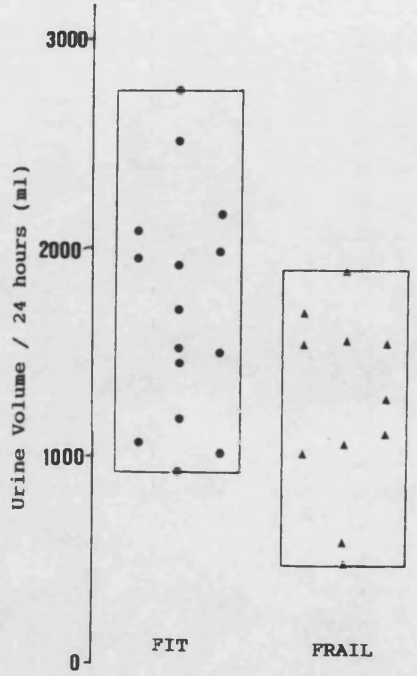


Fig 4.4.3 Distribution of Urine Volume / 24 hours in Fit & Frail Elderly People Taking FRUMIL



● = fit subject ▲ = frail subject

Fig. 4.4.4 Distribution of %Du in Fit & Frail Elderly People taking FRUMIL

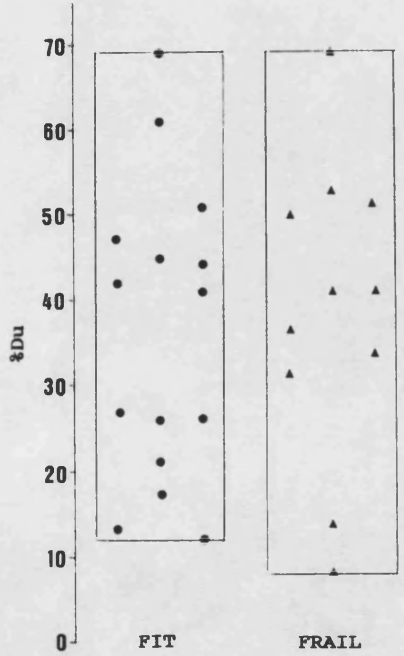


Fig. 4.4.5 Distribution of FRU Cls in Fit & Frail Elderly People taking FRUMIL

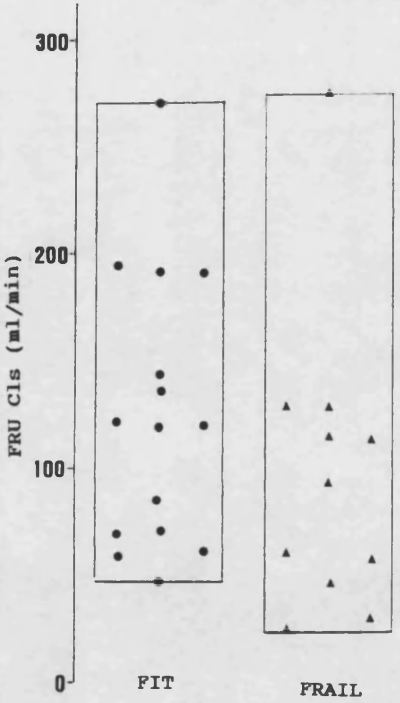
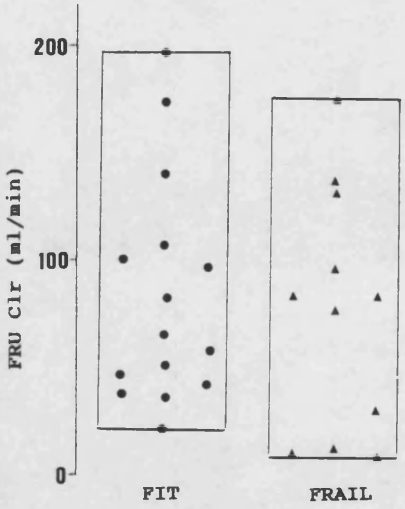


Fig. 4.4.6 Distribution of FRU Clr in Fit & Frail Elderly People taking FRUMIL



● = fit subject

▲ = frail subject

4.5 DISCUSSION

In this study the elimination of FRU was investigated in elderly people chronically taking FS or FM, with varying degrees of immobility & frailty. FRU excretion was followed to determine whether immobility or frailty appeared to influence the efficiency of elimination.

4.5.1 Frusemide Kinetics

17 fit & 18 frail subjects with mobility scores ranging from 1 to 4 took part in the study. 25 were taking FM, and of those 14 were fit. FRU kinetics were followed from 3-24h post-dose and over this period underwent first-order kinetics, with log serum [FRU] v time yielding a linear plot. This agrees with other studies where FRU disappearance has been described by an open two compartment model, with the biexponential decline in serum [FRU] resolving into 2 first-order disposition constants (53,56, 57,58). The first phase predominantly describes distribution and has a half-life approximating to 10m, with peak serum concentrations occurring about 60 minutes after oral dosing (53,60,189). The longer second phase corresponds to drug elimination whose $t_{1/2}$ is estimated to be 50-70 minutes in controls (48).

Only the elimination phase was followed in the present study, since this involved fewer blood samples being taken. Sampling times were therefore chosen such that distribution would be complete and peak plasma concentration passed before sampling. Analysis of results showed that blood sampling times were not optimal and would have been more consistent had samples been taken earlier and closer together. An improved scheme for sampling would have been 2,3,4,5 & 6h post-dose. Correlation coefficient of log

serum [FRU] v time, used to calculate $t_{1/2}$, varied between 0.997-0.821 (0.942±0.05; mean±s.d.) and in a few cases $t_{1/2}$ had to be calculated from 2 time points. An initial pilot study would have been advantageous.

No statistical difference was seen between sexes for $t_{1/2}$ or Cl, for FS or FM. This agrees with other studies which have found that FRU excretion is not influenced by sex (56, 58,62). The groups discussed were therefore of mixed sex.

4.5.2 Elimination Half-life

Elimination $t_{1/2}$ was determined in all but one subject taking FS (F49F) whose case will be discussed later. The range in $t_{1/2}$ for the remaining subjects was wide, from 0.82 - 11.90h (4.49±2.85) for the group taking FM & 1.36 - 20.17h (9.83±5.55) for those taking FS.

Means & ranges are greater than those reported from investigations of FRU excretion in young healthy individuals where mean $t_{1/2}$ has been estimated as 52±15, 36±5, 51±4 & 70±16 minutes when FRU was assayed by HPLC (61 190,191,192). Earlier work on FRU disposition involving paper chromatography, spectrofluorometry or radiolabelling are thought to have been less specific; using these methods mean $t_{1/2}$ estimates have ranged from 26-72 minutes (48).

Studies of FRU elimination in particular disease states have found a wider variation in mean $t_{1/2}$; patients with uraemia were found to have a 3 fold prolongation in $t_{1/2}$ compared to controls (51±4m controls, 156±25m uraemics; $p<0.05$) although in the same study (59) patients with nephrotic syndrome were found to have a similar $t_{1/2}$ to controls (54±6m). Another study compared FRU $t_{1/2}$ in those suffering from renal failure with controls - $t_{1/2}$ averaged

0.79h in controls & ranged from 1.15-24.58h in patients (61). While the longest $t_{1/2}$s were found in those with advanced renal disease, 2 patients with CCr of 8 & 6ml/min had $t_{1/2}$s of 1.42 & 1.55h, comparable with controls. Investigations into FRU disposition in patients with acute pulmonary oedema found that elimination $t_{1/2}$ varied widely from 127-1190m and presence of acute MI or differences in the severity of pulmonary oedema did not correlate with variations in $t_{1/2}$ (58). Patients with heart failure were also found to have an increased elimination $t_{1/2}$ compared to normals (60). Patients with decompensated heart failure not previously treated with FRU had a mean $t_{1/2}$ of 92m while those with cardiac decompensation under long term FRU treatment had a mean $t_{1/2}$ of 134m.

Studies of FRU disposition in old age have concluded that increased age appears to prolong elimination $t_{1/2}$, although to what extent is uncertain. When FRU was administered to healthy "elderly" volunteers (age 64±4y), mean $t_{1/2}$ was 102 ±33m (62-149m), compared to 70±20m seen in healthy young volunteers (57). A second study (62) used elderly patients (70-93y) already on FRU long term for chronic disease, and found $t_{1/2}$ to range from 59-317m.

Elimination $t_{1/2}$ obtained in the present study are greater than those seen in healthy young & elderly volunteers, but agree with those obtained in patients suffering from cardiovascular disease or chronic renal impairment. All subjects used in the present study were already taking FRU for treatment of a specific disease state, that is, no "healthy" volunteers were used. The fit & frail definitions are relative terms, and fit subjects were far less disabled by their illness than frail subjects.

FRU $t_{1/2}$ has been reported as increasing in old age, although the degree by which it changes is not certain (57, 62). The present study aimed to determine whether frailty or immobility influenced FRU elimination and so the age range of subjects studied was relatively narrow. Absence of correlation between $t_{1/2}$ & age for those taking FM is not contrary to previous studies since the majority of subjects were aged between 70-86y. Mean $t_{1/2}$ was 4.31±3.73h for those aged 70-78y & 4.40±2.02h for those aged 80-86y. Only 6 subjects taking FM were outside the age ranges. Similarity of mean $t_{1/2}$ between the cohorts explains the lack of correlation seen between age & $t_{1/2}$ in the FM group For subjects taking FS, a tendency towards a correlation between age & $t_{1/2}$ was seen. For those aged 67-69y $t_{1/2}$ was 5.30±2.64h & for those aged 81-89y the mean was 15.49± 4.72h; 2 subjects were outside these ranges, aged 78 & 89y. The difference in $t_{1/2}$ between cohorts gives rise to the trend towards a correlation between age & $t_{1/2}$ despite the small age range and limited numbers used. However, all subjects aged 81-89y were frail while of those aged 67-69y 2 were fit & 2 frail. The correlation between age & $t_{1/2}$ may have arisen due to the loss of fitness associated with an increase in age, rather than age per se.

No correlations were seen between $t_{1/2}$ & SCr, CCr or CCr/SA FRU is highly protein bound (approximately 98%) and so elimination is mainly via active secretion into the kidney tubule lumen via the non-specific organic acid secretory pathway (194), since only free drug can be excreted by glomerular filtration (48). Both GFR & tubular secretion are known to decline with age although controversy exists over whether the rates are linked. The reduction in GFR

with age, even in the absence of significant pathology, is now well documented (123,136) and observed in this study for all participating subjects (age v CCr; $p<0.02$).
Less is known about the decline in tubular function with age, since it is much more difficult to measure. Tubular mass has been show to decline with age but the loss of tubular function is not a homogeneous process and not associated with a commensurate contraction of kidney mass. The question of whether the nephron degenerates as a unit is unresolved. One study (195) concluded that a significant correlation between dimensions of the glomerulus & proximal tubules in young subjects was lost as the kidney aged. Hypertrophy of tubules in nephrons with normal or even absent glomeruli was observed. Conversely, other workers who examined 100 kidneys from subjects who died suddenly showed that after the fourth decade the volume of glomeruli & tubules diminished at the same rate (196). Since the majority of FRU is excreted by tubular secretion, $t_{1/2}$ would not necessarily correlate with CCr if proximal tubules & glomeruli degenerated at differing rates.
A plot of CCr v $t_{1/2}$ shows that for those taking FM or FS in this study, subjects with compromised renal function can have a prolonged or normal $t_{1/2}$ compared to those of a similar age with good renal function. Some studies have shown a direct correlation to exist between CCr & $t_{1/2}$ (58) while others have not (59,61). The variation in $t_{1/2}$ may be explained by differences in rate of tubular & glomerular degeneration which may occur in old age & renal impairment. In renal failure endogenous anions may accumulate which can compete with anionic drugs for the active anion secretory pathway (59). Variations in concentrations of endogenous

anions may influence the amount of FRU excreted by the tubules. Alternatively, the diversity of $t_{1/2}$ could be due to differences in nonrenal clearance (61), discussed presently.

Differences could also be due to changes in Vd or body composition between individuals. Elimination $t_{1/2}$ is a hybrid quantity, depending on both clearance & Vd. Since $t_{1/2}$ is inversely proportional to Cl, any change in Cl is only reflected in an increased $t_{1/2}$ if Vd is not significantly altered in those being compared. Age is known to be associated with alterations in body composition, which in turn affect Vd (9). A decline in lean body mass & increase in adipose-tissue in relation to total body mass is generally seen in old age (10,108,197), and variations in their proportions between subjects could influence Vd and hence $t_{1/2}$ in this study. Weight & SA did not correlate with $t_{1/2}$ for FM or FS, and these are known to influence Vd, but do not reflect body composition.

The apparent Vd of an extensively protein-bound drug may be influenced by changes in the extent of binding to plasma proteins (9,18). Studies have consistently shown an age-related decline in albumin, which appears to be the only plasma protein with which FRU binds (52). The reduction in albumin may be large when elderly subjects are poorly nourished, have advanced or chronic illness or are severely debilitated (49). Studies have shown the percentual binding of FRU to albumin to be slightly but significantly reduced in serum from elderly patients than in serum from controls (96.5±0.7% v 97.7±0.3%) with a positive correlation between protein binding and albumin concentration (50). Decreased binding has also been found in acute renal failure (51,52),

nephrotic syndrome & ureamia (59) and in anephric patients (56). The age & disease-related changes in the free fraction of FRU could influence Vd to complicate interpretation of the differences in elimination $t_{1/2}$ observed between subjects.

One frail subject (F49F) taking FS had only one measurable serum [FRU], at 1.92h, with a low recovery of FRU in urine (8.6%). The subject was obese (84kg), oedematous, had a CCr of 55ml/min and was not taking any other drugs concomitantly which were known to influence FRU absorption or elimination. Although it is possible that the daily dose of FRU was not taken, and that FRU detected was from a late dose the previous day, this is unlikely since the patient was hospitalized. These observations suggest that either reduced absorption or increased metabolism were occurring. Although no other person had only one detectable serum [FRU], a wide variation in $t_{1/2}$ was seen between subjects which cannot be accounted for by changes in CCr as discussed above.

Age **per se** has not been reported as influencing drug absorption except in a very few specific cases. Early work carried out on kinetics in the elderly found absorption of sulphamethizole, phenylbutazone & paracetamol unchanged (13). Later reports agreed with this finding, including one where absorption of FRU was investigated and found to be only very slightly decreased in old age (55). However, reports with regards to FRU absorption in disease states have varied, and it is thought that reduced absorption may in certain cases be responsible for "diuretic resistance". This term has been used to describe cases where even large doses of oral diuretic have evoked a very small response

(43). One case was reported of a patient with ideopathic oedema who exhibited a change in FRU bioavailability according to the degree of oedema - it varied from 75% in the oedema-free to only 17% in the oedematous state. Kinetic parameters were similar in both states and response to i.v. FRU was unchanged (198). It was proposed that the changes were due to reduced absorption from the gastrointestinal tract which was affected by oedema. Another group reported erratic and incomplete absorption of FRU in CHF (199). Patients with severe CHF were given oral & i.v. doses of FRU & AUC ratios compared. Average absorption was 61%, consistent with previous reports (48), but varied widely from patient to patient (31-79%). Kinetics were again comparable between patients. Another study reported no difference in FRU bioavailability between patients with CCF & controls, although again the interindividual variation was wide but the within subject variation was low (200). Other studies have reported FRU bioavailability to vary from 34-80% in heart failure (48) with a decrease also seen in patients with uraemia or nephrotic syndrome. Reports have also suggested than FRU is ineffective orally when CCr is less than 3ml/min thought to again be due to impaired absorption from the GI tract since i.v. FRU remains effective (43,201). Conflicting results were obtained by another group of workers investigating FRU absorption in decompensated heart failure (54). Each subject served as their own control, with FRU absorption compared before and after cardiac compensation. While lag time & time to peak serum concentrations decreased by 57% & 27%, and peak serum [FRU] increased by 27%, no significant changes occurred for absorption or

elimination $t_{1/2}$s, AUC or absorption of D-xylose, when the two states were compared. This suggests a qualitative but not quantitative, alteration in FRU absorption in decompensated heart failure. This was thought to be a contributing factor to diuretic resistance seen in some patients.

There have also been reports of malabsorption of FRU by phenytoin, which can reduce absorption by 50%, with a corresponding reduction in C_{max} but unchanged clearances. The mechanism for malabsorption is not clear (202).

It is possible that reduced FRU absorption could account for a low serum [FRU] & %Du in subjects such as F49F but no reports have found an associated change in kinetics. This would imply that even if reduced absorption occurred in some cases it could not account for the variation in $t_{1/2}$.

Mobility score was not correlated with $t_{1/2}$ suggesting that mobility is unlikely to be an important determinant of FRU excretion rate. From these results it would appear that while age & probably clinical condition may influence FRU elimination $t_{1/2}$, no direct correlations exist which can be used to predict $t_{1/2}$ for a particular subject. Other factors are important and will be discussed in 4.5.3.

Subjects taking FS were found to have a significantly greater $t_{1/2}$ than those FM - when all $t_{1/2}$s were ranked, subjects taking FS were responsible for 5 out of 6 highest results. It seems that either the presence of amiloride in FM influences FRU disposition, or those subjects prescribed FS are in some way different from those prescribed FM. Considering the 10 subjects taking FS, more were frail (n=7) than fit (n=3). They tended to pass less urine than

the FM group despite the dose of FRU being greater for some FS individuals. Fluid intake was not measured in either group. While no significant differences were seen between clearances of the groups, inspection of data showed that the lower quartile was consistently lower, and s.d. greater, in the FS group for Cls, Cls/kg & Clr. FM & FS groups were similar in age, mobility, UCr, SCr, CCr & CCr/SA.

Two frail subjects (F14GPF & F51F) taking FS were also taking spironolactone but their $t_{1/2}$s did not appear to differ from those only taking FS. Other workers have reported a lack of pharmacokinetic interaction between spironolactone and FRU, although a trend towards a higher clearance and lower $t_{1/2}$ for FRU was seen (203); if spironolactone influenced FRU excretion it would have been in the opposite direction to that observed between FS & FM groups. With only two subjects taking spironolactone a definitive statement cannot be made but there appears to be no need to exclude them from the FS data.

Two fit subjects (F05GPF & M17GPF) taking FM were also taking a non steroidal anti-inflammatory drug (NSAID) concomitantly. An interaction has been reported to occur between indomethacin & FRU, probably due to inhibition of prostaglandin synthesis (42,43), resulting in a loss of response to FRU and possible reduction in FRU renal clearance. The two subjects taking a NSAID had relatively high $t_{1/2}$s but M17GPF had a low CCr. If an interaction had occurred in these subjects, the effect would be an increased $t_{1/2}$. Since those taking FM have a significantly lower $t_{1/2}$ the use of a NSAID by two subjects in the FM cannot explain the result. Amiloride is a potassium

sparing diuretic seldom used alone, but frequently administered in combination with thiazide or loop diuretics to attenuate their potassium wasting effects. It is a basic drug eliminated by filtration and active secretion via the nonspecific organic base secretory pathway of the proximal tubule. A study of amiloride disposition in the elderly has shown that age appears to reduce clearance and increase $t_{1/2}$, probably due to a reduced GFR observed in old age (204).

No studies have compared FRU disposition when alone and in combination with amiloride, and so the possibility of a pharmacokinetic interaction between FRU & amiloride during absorption, distribution or elimination cannot be ruled out. Amiloride is unlikely to influence FRU absorption since it is not related to any drug known to do so. Albumin has high capacity but low affinity binding for most cationic drugs, with a low capacity but high affinity for acidic drugs. There are no reports of amiloride binding to albumin making this mode of interaction also unlikely. FRU is secreted by the organic acid pathway while amiloride is secreted by the organic base pathway, so no competition would be likely at that site.

It is difficult to explain the difference observed between FM & FS groups on the basis of a pharmacokinetic interaction, although the possibility remains. A more likely explanation may be the preponderance of LVF observed in the FS group when compared to those taking FM. This difference in pathology between the groups may account for the reduced efficiency of elimination in the FS group. From these results it seems likely that there is a difference between those taking FS & FM despite comparable

kidney function & mobility, and thus more appropriate to treat the groups separately than to combine data.

4.5.3 Apparent Serum Clearance of Frusemide

To determine a drugs total body clearance i.v. administration is necessary. This was not possible and so oral FRU was given and apparent (relative) serum clearance (Cls) was calculated. FRU is rapidly but incompletely absorbed from the gastro-intestinal tract, with 50-70% of an oral dose being available for systemic circulation (48, 53,193,199,205). A figure of 50% of the dose administered was used to calculate Cls in this study, it being assumed that absorption was similar for all individuals studied. Studies have shown that while age does not appear to significantly alter FRU absorption (55,62), bioavailability of FRU may be altered in some disease states as discussed in 4.5.2. All subjects studied were elderly and clinically stable and whilst the percentage of dose recovered in urine varied between subjects, no significant difference was seen between fit & frail groups. These results suggest that the assumption is reasonable, although it could only be proven with comparative i.v. and oral studies.

Using a figure of 50% systemic bioavailability, Cls ranged from 16-279ml/min (116±69) in the FM & 14-422ml/min (115± 139) in the FS group. Cls/kg ranged from 0.31-4.36ml/min/kg (1.72±0.99) in the FM & 0.23-6.59ml/min/kg (1.90±2.09) in the FS group. Other workers have calculated Cls following i.v. FRU and obtained different values according to the method of analysis. When HPLC has been employed, Cls in young healthy volunteers has been estimated at 194±35, 170±19, 219±49 & 170±19ml/min (56,57,61), 2.18±0.12 & 2.54± 0.96ml/min/kg (59,206). Studies of FRU excretion in old age

have calculated Cls to be 129±11ml/min in healthy elderly subjects (64±4y) & 73±27 ml/min in elderly patients with cardiovascular disease on long term FRU therapy (57,62). Cls has been reported to be 86±16ml/min in patients with cardiac decompensation previously untreated with FRU & 69± 22ml/min in patients with cardiac decompensation under long term FRU therapy (60). Cls in renal failure has also been investigated, and estimated to be 66±19ml/min in anephric patients, 2.96±0.51 & 2.54±0.96ml/min/kg & 80±29ml/min in those with nephrotic syndrome (56,59,61,206). Patients with uraemia have been reported to have Cls of 0.62±0.1ml/min/kg (59). Estimates of Cls in the present study are lower than those seen in healthy young volunteers, but are comparable with those for elderly and chronically ill subjects. There is a wide interpatient variation however, with a very high Cls being seen in a few individuals.

For subjects taking FM or FS, Cls correlated with CCr & CCr/SA in agreement with findings from other studies (58,59 61). While renal function appears to be a determinant of FRU Cl, it is not the only one, since a few subjects with low CCr had a high Cls. GFR is known to decline with age and this could lead to the reduction in Cls observed in those studies carried out in elderly subjects. The importance of renal function in FRU excretion would also explain the reduction in Cls seen in patients with nephrotic syndrome or uraemia. Reasons for the interindividual variations seen will be discussed in 4.5.4.

4.5.4 Renal Clearance of Frusemide

Clr in the present study was calculated to be 82±55ml/min (1.24±0.85ml/min/kg) for subjects taking FM and 79±91ml/min

(1.37±1.40ml/min/kg) for those taking FS. This agrees with other groups investigating FRU Clr in elderly & chronically ill patients. A mean Clr of 75±9ml/min was found for healthy elderly (57), 40±18ml/min for chronically ill elderly (62), 1.18±0.29 & 1.31±0.59ml/min/kg in patients with nephrotic syndrome (59,206), & 0.06±0.01ml/min/kg in uraemics (59). FRU Clr is generally agreed to be reduced in old age & chronic illness compared to healthy young volunteers.

There was a tendency for Clr to be correlated with CCr in both the FM & FS groups. This has been observed in other studies (62,48) but not all (58,59). Where a correlation has been found, it is thought to be due to the role renal function plays in FRU excretion in urine, when tubular secretion decreases with GFR. However, this correlation is not seen in all studies, suggesting that other routes of elimination besides renal excretion are possible.

In severe renal impairment accumulation of endogenous anions may inhibit active anion transport and thus reduce Clr of drugs such as FRU (59).

When renal clearance is subtracted from total body clearance the product represents that FRU cleared by nonrenal routes. In this study nonrenal clearance, Cl_{nr}, could not be calculated since oral administration only enabled Cls to be estimated, but Cls & Clr were correlated. Studies have found Cl_{nr} to approximate to 42% of Cls in healthy elderly (57) and 47% in the chronically ill elderly on long term FRU (62). In uraemia Cl_{nr} was reduced by 41% of that seen in normals but in nephrotic syndrome it tended to increase (59), as confirmed in a second study (206). Controversy exists about nonrenal routes of FRU excretion.

Radiolabelling studies have detected FRU in bile which may be one route of elimination, increasing when renal function is impaired (53,61). It has been suggested that FRU is metabolized to FRU-glucuronide & 4-chlor-5-sulphamoyl-anthranilic-acid (58,60,62). The glucuronide conjugate has been detected in urine of patients administered FRU, with elderly appearing to excrete less than young volunteers (57 62). The amount of glucuronide excreted has been reported to vary from 0.7mg/24h in subjects not previously treated with FRU, to 7mg/24h in subjects given FRU for a minimum of 6 months, when a 40mg dose is given i.v. (60). This has given rise to the idea that chronic FRU therapy may induce glucuronidation, to enable $t_{1/2}$ to be normal despite a reduced CCr. With the advent of HPLC, while FRU-glucuronide has been detected, CSA has not. Any remaining FRU not excreted renally, secreted into bile or metabolized to FRU-glucuronide may be metabolized by another route but evidence is lacking.

It is possible that the degree of nonrenal elimination may have varied between subjects in the present study. Elimination $t_{1/2}$ did not correlate with CCr suggesting that filtration alone is not responsible for FRU excretion. If tubular secretion declines at a similar rate to GFR, the lack of correlation suggests that extrarenal elimination is taking place. The wide interindividual variation in both $t_{1/2}$ and clearance also suggests that nonrenal excretion may be occurring, to differing degrees in those studied. Subjects on long term FRU may have developed an enhanced glucuronidation pathway which could give rise to a near normal $t_{1/2}$ in some with low CCr. Clearance is calculated assuming 50% bioavailability but reports indicate that this

may change with clinical condition, and this could explain the variation in Cl. Elimination $t_{1/2}$ is not calculated using dose absorbed and yet a wide variation is also seen with this parameter. It seems likely that extrarenal metabolism has occurred in some subjects studied.

4.5.5 Urine Volume & Frusemide Dose

Urine volume passed over 24h ranged from 472-2761ml (1515±564) for subjects taking FM & 656-3217ml (1450±819) for those taking FS. Volumes were lower than would be expected following a potent diuretic, suggesting that some subjects were fluid-depleted. Urine volume was not related to FRU dose for either group, whilst urine volume correlated with Cls for subjects taking FS.

4.5.6 Frusemide Recovery in Urine

All fit subjects taking FM took 40mg FRU & frail subjects took 40 or 80mg FRU. Of those taking FS, all fit subjects took 40mg & frail subjects took 40,80 or 120mg FRU. The absolute amount of FRU in urine could not be compared & so percent of dose recovered in urine was calculated (%Du). Means & ranges of %Du were similar for both groups - subjects taking FM excreted from 8-69% (36±16) & those taking FS excreted 9-65% (33±15).

Elderly patients have been reported as excreting 22-55% of an oral dose in urine as FRU & FRU-glucuronide, with recovery increasing to 51-84% after i.v. injection (62). Urinary recovery of an oral dose was reported to drop to 1.4% & 0.7% in patients with CCr of 3ml/min (61).

One subject taking FS & 5 taking FM excreted more than 50% of dose administered. This suggests that in some cases in excess of 50% of the dose is absorbed although this figure

was used in clearance calculations.
The wide interpatient range of %Du suggests that either absorption is not comparable or nonrenal excretion differs between subjects. FRU metabolites were not assayed and so it is not possible to say from the data which is the most likely. Studies report a change in both absorption and nonrenal excretion in certain disease states.
It is possible that urine was not collected accurately for 24h, and this gave the wide variation in %Du, but this would only account for those subjects excreting lower than expected amounts. The urine collection could have been carried out for longer than 24h but this would have involved the omission of at least one dose of FRU which would not have been acceptable to many of the participants. A collection period in excess of 24h would also have caused more inconvenience for those subjects who were mobile, and while this possibility was examined it was not felt to be practicable particularly in the community.
Subjects with the shortest $t_{1/2}$ did not excrete significantly more of the dose, also suggesting that a longer collection period would not have increased recovery. %Du was related to CCr for both groups but this relationship failed to reach significance. FRU excretion is only partially dependent on GFR as previously discussed, and this route of elimination appears to become less important with a decline in renal function. This could explain the weak correlation seen.

4.6 FRUSEMIDE KINETICS IN FIT & FRAIL ELDERLY PEOPLE

4.6.1 Comparison of Age

14 fit & 11 frail subjects took FM. Age was significantly

different between groups, with the fit being on average 8y younger than the frail. This arose from a scarcity of "young" immobile volunteers - only 2 subjects below 80y were frail. It would have been possible to recruit more immobile subjects if the study been extended to more than one general practice. At the time this was not possible but this method was later used in the digoxin study where recruitment was difficult. A sub-group was formed of FM subjects in the age range 76-86y in an attempt to separate the influence of age and frailty on parameters measured. This will be discussed in 4.6.8

3 fit & 7 frail subjects took FS and age was not significantly different between groups.

4.6.2 Mobility Score

Mobility score was significantly different between fit & frail groups as expected since fit & frail definitions included information on mobility. For those taking FM there was little overlap of scores between groups - 1 fit & 5 frail subjects had a mobility score of 3. No overlap was seen for the FS group - all fit subjects had a score of 1.

4.6.3 Comparison of Elimination Half-lives

For those subjects taking FM, no significant difference was found for $t_{1/2}$ between fit & frail groups despite the age difference, although the trend was towards a reduced $t_{1/2}$ in fit subjects (4.00±3.33h fit, 5.12±2.06h fr). The range of $t_{1/2}$ was greater in the fit group with 3 subjects (F05GPF, M08GPF & M10GPF) having $t_{1/2}$s of 9.13, 11.90 & 7.56h. These subjects all had mobility scores of 1, and CCr of 55, 29 & 77ml/min respectively. The high $t_{1/2}$ & reduced CCr of M08GPF were probably linked, but the CCr of

the other 2 subjects were normal for their age and cannot explain the $t_{1/2}$ - M10GPF had the highest CCr of the entire group. None were on drugs known to interact with FRU disposition. This would suggest that neither age or CCr can be used to predict FRU $t_{1/2}$ in the elderly. Fitness and clinical condition may be important, but all 3 subjects were highly mobile at the time of the study. However, 22 months after the study, both M08GPF & M10GPF were deceased, although the cause of death was not known. Unfortunately, overall mortality rates in fit & frail groups were not known. Subjects were categorized as fit or frail according to a combination of factors detectable by eye. No biochemical or haematological tests were carried out since this was thought to be more relevant and applicable to the situation faced by the prescriber. Had blood tests been carried out it is possible that undiagnosed pathology may have been detected in those subjects now deceased which may have led to them being categorized as frail rather than fit. If fit & frail definitions included results from blood tests the usefulness would be limited, particularly in general practice.

For those taking FS, 3 were fit & 7 frail. Fit subjects had a smaller range, a lower mean $t_{1/2}$ and smaller s.d. than frail subjects but these observations failed to reach significance. One fit subject (F22GPF) recruited from general practice had subsequently died. Again the disparity between $t_{1/2}$ & CCr can be seen - one fit subject with a CCr of 45ml/min had a $t_{1/2}$ of 9.33h and another with a CCr of 50ml/min had a $t_{1/2}$ of 1.36h. Other factors besides CCr and mobility appear to be important in the determination of $t_{1/2}$.

As previously discussed, elimination $t_{1/2}$ is a hybrid function of both Cl & Vd. Groups were matched for weight & SA but body composition & hence Vd may have differed between fit & frail subjects. However, the assumption that absorption is comparable for all subjects, made for Cl calculation, may not be correct. Differences seen between the fit & frail for $t_{1/2}$ is therefore important since $t_{1/2}$ is calculated without the inclusion of % dose absorbed.

4.6.4 Comparison of Apparent Serum Clearance

For those taking FM, Cls ranged from 47-273ml/min (130±64) for fit & 16-279ml/min (98±64) for frail subjects ($p<0.2$). Although the ranges are similar for both groups there is a trend towards an increased Cls in the fit group.

The fit had a significantly greater CCr than the frail group ($p<0.02$). If Cls v CCr is plotted for fit & frail a relationship between them can be seen with a few notable exceptions. One frail subject (F47F) had a CCr of 33ml/min but a Cls of 278ml/min. This subject is separate from the rest of the frail group but the reason for this is not clear. The category she was placed in was correct considering her clinical condition.

Results indicate that other factors besides CCr and fitness category appear to influence Cls. It is possible that the assumption made about absorption in the calculation of Cls may not be correct, and that differences in absorption may have given rise to the differences in Cls.

For the FS group Cls of fit varied from 39-422ml/min (187± 206) & 14-263ml/min (78±96) for frail subjects ($p<0.4$). The results show that fit subjects tended to have greater Cls, but there were only 3 subjects in that group, and the

s.d. was wide. However, one frail subject with a CCr of 28ml/min had a Cls of 263ml/min which emphasises the difficulty in determining what factors influence FRU Cls. When Cls was expressed per kg bodyweight the differences between fit and frail groups observed remained the same.

4.6.5 Comparison of Renal Clearance

For those taking FM, Clr ranged from 22-197ml/min (85±55) in fit & 10-176ml/min (78±57) in frail subjects ($p<0.7$). The fit group had higher minimum, maximum, Q1 and mean values for Cls, but Q3 and median were greater for the frail group. While CCr correlated with Cls for the FM group, this was not the case for Clr. Subjects with a low CCr had some of the highest Clr values. A similar pattern was seen for Clr/kg when the fit and frail groups were compared.

CCr had been calculated over 24h and also over 3 time periods each approximating to 6-8h. The CCr 0-6h was compared with the Clr 0-6h and with a few exceptions the pattern seemed to be more predictable, and the correlation was significant when all those taking FM were considered ($p<0.01$). CCr 0-6h appears to be a more reliable predictor of FRU Clr, but other factors are also important.

For the FS group, Clr ranged from 24-289ml/min (124±144) for the fit & 11-109ml/min (52±40) for the frail group. Clr was higher for the fit group but there were only 3 subjects in that group. When CCr 0-6h was plotted against Clr 0-6h a relationship was seen but was not significant.

4.6.6 Comparison of Urine Volume & % Dose Excreted in Urine

For the FM group, urine volume ranged from 928-2761ml (1727 ±567) for the fit & 472-1880ml (1245±449) for the frail

group ($p<0.06$). Volumes were low suggesting that some subjects were fluid depleted. One fit subject and 2 frail subjects passed volumes less than 1000ml but these subjects did not excrete the lowest % dose in urine, suggesting that urine collections were accurate. Frail subjects tended to pass lower volumes despite being administered on average a greater dose of FRU. However, measurement of fluid intake was not attempted in either group, although this may have provided some useful information on accuracy of urine collection.

%Du ranged from 12-61% (34±15) for the fit & 8-69% (39±17) for the frail group, with the similarity in %Du suggesting a comparable accuracy of urine collection between groups. However, there was no correlation between FRU dose & urine volume. The dose response curve for FRU is not linear and there is a ceiling response - if the dose of FRU is doubled the diuresis would not necessarily also double (48).
It is possible that subjects with a low urine output poorly absorbed the FRU or suffered circulatory fluid depletion which could contribute to a reduced urine output. Elderly patients have an impaired capacity to reabsorb water, manifested by a reduction in maximum concentrating ability (24,207), and this can readily lead to dehydration, both from lack of water and sodium. Diuretic use can upset the normal physiological state and hospital routine may compound this problem (40,139). This can lead to fluid depletion which may account for the reduced urine volumes.

For the FS group, urine volume ranged from 1093-1458ml (1245±190) for the fit & 656-3217ml (1553±1010) for the frail group. %Du varied from 29-34% (32±2) in the fit &

9-65% (33±19) in the frail. There appears to be little difference between the fit & frail groups taking FS in terms of response to, or excretion of FRU in urine.

4.6.7 Age-Matched Subjects Taking Frumil

Subjects taking FM aged between 76-86y were selected to form fit & frail subgroups, to enable the influences of age & frailty to be separated, and appendix D16 gives the results. When fit (n=8) & frail (n=7) groups no longer differed in age, mobility score remained significantly different. UCr, SCr, CCr, weight, SA, %Du & urine volume were similar between groups.

Both Cls & Clr tended to be lower in the frail group, but this was not significant, and this trend disappeared when Cl was normalized for bodyweight. However, elimination $t_{1/2}$ was significantly higher in the frail group, with the mean value differing by 1.4h.

4.7 CONCLUSIONS

The elimination of FRU was followed in fit & frail elderly people with chronic illness of cardiovascular origin, with the aim of determining whether mobility & "fitness" influenced FRU excretion. FRU had to be administered orally, and blood samples were not taken at optimum time intervals. These limitations meant that data was interpreted with caution.

Elimination $t_{1/2}$ was calculated by estimating the gradient of time v log serum [FRU] plot. Blood sampling was not optimal with the correlation coefficient of the line varying; in a few cases $t_{1/2}$ was calculated from two time points. Elimination $t_{1/2}$ is a hybrid quantity, depending

on both clearance and Vd. Vd is in turn influenced by weight, body composition, and plasma-protein binding, all of which can change in old age & chronic disability. There are inherent errors in the accuracy of FRU analysis by HPLC, although the coefficient of variation was low (3.1% at 0.1ug/ml, 2.3% at 2.0ug/ml), and further difficulties were experienced in the collection & assay of a light-sensitive compound. In order to calculate apparent clearance it was assumed that absorption was comparable for all subjects studied. However, a review of the literature showed that the assumption may be invalid in some cases. Furthermore, groups of subjects were small and heterogeneous. Because of flaws in this study comparisons made should be considered orientating & qualitative rather than definitive & quantitative.

All participants were regularly taking FRU for a variety of cardiovascular problems. None were "healthy" volunteers and the terms "fit" and "frail" were therefore used relatively in this study, according to the definitions given in appendix A1. The lifestyle of fit individuals appeared to be relatively unaffected by the presence of chronic disease and all were able to live independently in the community. On the other hand frail subjects experienced some degree of disablement through chronic illness, and although none were acutely ill, all were unable to live independently, requiring assistance with activities of daily living. Those classified as frail were also likely to have more significant pathology, and be taking more drugs. The definitions did not require biochemical or haematological tests to be carried out since this was thought to best represent the situation usually faced by

the prescriber. Had a battery of tests been run it is possible that some fit subjects may have been recategorized as frail. This would have had the effect of increasing numbers in the frail group but the trends observed would have most likely remained the same.

Subjects taking FS appeared to eliminate FRU less efficiently than those taking FM. While there was no statistical difference between subjects taking FS & FM with respect to CCr or mobility score, those subjects taking FS were more likely to be frail. The decision to prescribe FS rather than FM may have been made on the basis of diagnosis (more subjects taking FS had LVF), or results from biochemical tests obtained by the physician. Since there appeared to be a difference between those taking FM & FS the groups were kept separate rather than combined which had been the original intention. Despite the reduced numbers seen in FS and FM, both exhibited similar patterns.

Since the calculation of $t_{1/2}$ in some cases involved the use of only two time points, and estimation of clearance necessitated the use of an unsubstantiated assumption, the findings from both will be combined to form a general statement.

Although significance was rarely reached, there appeared to be a trend towards a decreased elimination rate of FRU in frail subjects compared to their fit counterparts, and this trend remained when FM subjects were age-matched. While CCr tended to be related to clearance, $t_{1/2}$ was not. It is known that FRU is eliminated at least partially via the kidney; the minor fraction of free drug being excreted by glomerular filtration and the bound drug excreted via

secretion by proximal tubules (48). Studies have failed to show conclusively whether degeneration of tubules and glomeruli occurs at similar rates during the aging process (23). If rates are comparable, then the lack of correlation between elimination rate & CCr, remarkable in a few cases, suggests that either tubular secretion is inhibited or other excretory mechanisms are also responsible for the removal of FRU from the body.
In severe renal impairment, particularly with uraemia, endogenous anions may accumulate and compete with anionic drugs for secretion via the proximal tubule. If this occurred in some subjects it could account for those cases where Clr is especially low. Competition can occur to differing degrees, but this would not explain the apparently high Cls seen in some subjects with a low CCr.

Absorption of FRU following administration of a solid dose formulation is not complete (48), but even if systemic bioavailability approximated to 50% of the dose administered, recovery of unchanged FRU in urine was often substantially lower. Studies using i.v. administration have also showed a urinary recovery less than 100% (57,60). This suggests the existence of alternative pathways by which FRU may be eliminated, with the relative importance varying between individuals. Since the advent of HPLC, frusemide glucuronide, a metabolite of FRU, has been detected in urine (57,62), while the more controversial CSA has not. While it is not known whether other metabolic pathways exist, studies have shown that FRU may also be secreted into bile and excreted in the faeces (53,61).
In the present study only unchanged FRU in urine was assayed, and it is not possible to comment on whether

alternative metabolic pathways were responsible for the excretion of part of the FRU administered. However, those individuals with a low CCr who also had a low FRU $t_{1/2}$ may have eliminated FRU via other mechanisms besides renal clearance of unchanged FRU.

Frail subjects appeared to have consistently increased $t_{1/2}$s and reduced clearances compared to fit subjects, although this was only significant when subjects taking FM were age-matched. Frail subjects had a significantly lower CCr and this could be the explanation for the difference. Other studies have noted that while renal clearance is reduced in elderly compared to young volunteers the proportion of FRU cleared nonrenally is unchanged (57). If in the present study fit subjects excreted more FRU renally due to an increased GFR and accompanying increased tubular secretion, with both groups excreting similar quantities of FRU via nonrenal mechanisms this could explain the results. Fit & frail groups differed in many ways. The frail were much less mobile than the fit, spending more time sitting and supine. The difference in posture between the groups could explain the changes in FRU kinetics, since marked circulatory and plasma volume changes occur in the upright compared to the supine position (208). Benzylpenicillin, also eliminated via the nonspecific organic acid secretory pathway, has been shown to have an increased renal clearance but reduced first-order rate constant for metabolism when patients were subject to bedrest as opposed to normal activity. The authors concluded that bed rest per se could bring about pronounced changes in the distribution, metabolism and excretion of drugs relative to values obtained in ambulatory subjects (41,209). It was

thought that these changes, which have also been seen following physical exercise, could be due to an increased metabolic rate seen in active subjects.

Changes in posture may influence CCr, although evidence has been inconclusive. One study observed no change in CCr when subjects were rested in the antiorthostatic position compared to when they underwent ordinary ambulatory activities (167). However, a considerable increase in diuresis and sodium excretion was observed in the antiorthostatic position. When FRU was orally administered a summation of the effects of diuretic and antiorthostatic position was seen, thought to be due to different mechanisms of action. Another study found an increase in CCr, diuresis & natriuresis in the supine compared to a sitting position for "normals" and patients with hypoalbuminaemic states (168). CCr increased further when the subject was placed in the head down tilt position. Patients with cirrhosis and ascites given bumetanide i.v. have been found to have an increased GFR, and enhanced diuretic and natriuretic response when supine than when in the upright position (210). However, the kinetics of bumetanide in the different positions were not investigated.

In the present study frail patients, who were more likely to be inactive and spend longer in the supine position, had a lower CCr and reduced elimination of FRU. This suggests that the differences observed are not mainly due to differences in posture, although they may attenuate the effect of old age & frailty. To determine the effect of posture, a study could to be carried out with each subject acting as their own control and FRU administered on two

occasions with subjects either upright or supine. Frail subjects were less "well" than fit subjects although all were suffering from cardiovascular disease. Prolonged inactivity is known to cause gradual deterioration of cardiovascular function, and frail subjects may have a reduced cardiac output, stroke volume and renal plasma flow (57). These difference in clinical status between the two groups may have influenced FRU elimination.

To conclude, fit elderly people appeared to handle an oral dose of FRU more efficiently than their frail counterparts. Age appears to be associated with a decline in FRU elimination and this is partly related to the inevitable decline in kidney function seen with increasing age. In a few individuals impaired renal function, and hence reduced CCr, seemed to have little bearing on FRU elimination which was more efficient than would be expected. It would seem likely that FRU is eliminated by nonrenal routes, which may be enhanced in some individuals with poor renal function. The qualitative differences observed between fit and frail elderly people may in part be due to the inevitable differences in activity, posture and blood flow. More work needs to be done to investigate the probable routes of FRU metabolism in order to determine the cause of differences observed in this study.

CHAPTER FIVE

PARACETAMOL METABOLISM IN ELDERLY PEOPLE

5.1 INTRODUCTION

Paracetamol (PAR) is a commonly used mild analgesic whose elimination is accomplished by hepatic metabolism. A number of studies have been performed to investigate PAR disposition in both young & elderly healthy volunteers but little work has been done in the chronically ill, disabled elderly. In this study an acute dose of PAR (1g) was given to elderly people who were either "fit" (well & living independently in the community) or "frail" (having chronic illness, immobility & loss of independence). PAR excretion was followed to determine the influence of immobility & frailty.

5.2 PROCEDURE

Fit & frail elderly people aged over 64y were invited to take part in the study. Exclusion criteria are given in 2.2. Agreement of the volunteers' GP or Geriatrician was received before proceeding with the study. Each subject was provided in advance with an information sheet (appendix F1, standard or enlarged type) and able to question myself or Dr L Parker (trainee GP) prior to commencement of the study. Subjects avoided taking any PAR-containing preparation from the night before the study start.

On the morning of the study subjects ate a light breakfast of toast or cereal and an hour later emptied their bladder and took 2 x 500mg PAR tablets with a glass of water, either standing or sitting upright. Food was avoided for at least an hour after dosing, and thereafter taken freely.

During the study subjects were questioned about their day-to-day activities & mobility, categorized as fit or frail, and had their mobility scored according the mobility scale devised for the study (appendices A1 & A2). The form given in appendix F2 was filled in during the study when subjects were weighed & measured and any medications taken were noted. Demographic details, diagnoses & drugs taken are given in appendix F3.

5-10ml of blood was taken at 0,3,4,6 & 12h and all urine collected for 24 hours after dosing. PAR was measured in all samples, UCr in all urine samples, and SCr in serum at time 0. Urine samples collected over 24h were assayed in 3 aliquots each as near to 8 hours as possible with the final aliquot spanning the hours spent overnight in bed. Analytical methods for PAR & creatinine are given in 2.3.3.

For patients prescribed PAR regularly or "as required", further doses were omitted for 12h after the 1g test dose until the last blood sample was taken. After this time PAR was avoided wherever possible until completion of the study. If any patient required PAR after 12h then urine was voided before PAR was taken, to provide a final urine specimen for UPAR analysis. The urine collection continued for measurement of UCr excretion only.

PAR elimination half-life ($t_{1/2}$), AUC & apparent clearance (Cl) were calculated as per 2.4. From the urine data free urinary paracetamol (UPAR), PAR glucuronide + sulphate conjugates (UPARG+S), and total UPAR (free PAR + UPARG+S) were determined. SCr & UCr were measured to calculate CCr.

Correlations between parameters were determined using

Spearmans coefficient of rank correlation with significance levels taken to occur at $p<0.01$. Groups of fit & frail subjects and males & females were compared using the Mann-Whitney U test, with significance occurring at $p<0.05$.

5.3 RESULTS OF PHARMACOKINETIC PARAMETERS

5.3.1 All Subjects

56 subjects successfully participated in the study (34F & 22M). One patient (F37P) was later found to be taking phenytoin, known to interact with PAR metabolism, and so these results were excluded from statistical analyses (67, 211). Individual results are given in appendices F4 & F5. Results & correlations from the entire group (males + females) are summarized in appendices F6 & F7.

Age ranged from 64-97y (80±8; mean±s.d.) and was not related to M.Sc or $t_{1/2}$ but correlated with Cl, total UPAR, UCr, CCr & CCr/SA ($p<0.01$).

Mobility score correlated with CCr ($p<0.01$); UPARG+S, total UPAR & UCr ($p<0.001$).

PAR elimination $t_{1/2}$ correlated with Cl & Cl/kg ($p<0.01$).

A large inter-individual range was seen for Cl (5.3-38.7l/h a multiple of 7.3), which correlated with age, $t_{1/2}$, CCr & CCr/SA ($p<0.01$); urine volume, UCr, SA & weight ($p<0.001$). However, Cl/kg exhibited a smaller range (0.12-0.59l/h/kg; a multiple of 4.8) and only correlated with $t_{1/2}$ ($p<0.01$).

Urine volume correlated with age, UPARG+S, total UPAR, CCr & weight ($p<0.01$); Cl, UCr & SA ($p<0.001$).

Free UPAR showed no correlations. Between 0 (none detected) & 101.5mg of free UPAR (30.5±21.3), and 289.2-1192.1mg of total UPAR (715.1±185.3mg) were recovered in urine over 24h Of this an average of 680.8±184.8mg PAR equivalents (187.7-

1109.8mg) were as PAR glucuronide + sulphate. UPARG+S & total UPAR correlated with M.Sc & UCr ($p<0.001$); urine volume, age & CCr ($p<0.01$).
SCr ranged from 0.50-3.78mg/100ml (1.38±0.66), correlating with CCr/SA & CCr ($p<0.001$). CCr varied from 10-105ml/min (48±25) and CCr/SA from 11-115ml/min/1.73m^2 (51±24). Both correlated with age, M.Sc, Cl, UPARG+S, total UPAR ($p<0.01$) UCr, SCr, weight & SA ($p<0.001$).

5.3.2 Female Subjects

Results & correlations from all female subjects are given in appendices F8 & F9. The females were not well matched with respect to age & mobility, with no females below the age of 80 years having a mobility score of 3 or less. This gave a correlation between age & mobility score ($p<0.01$). Many of the relationships previously seen in 5.2.1 failed to reach significance when only the females were considered but the trends remained noticeable and formed a similar pattern to the entire group. Significant correlations were: age v M.Sc & urine vol ($p<0.01$); M.Sc v UCr, UPARG+S & total UPAR ($p<0.01$); $t_{1/2}$ v Cl ($p<0.01$); Cl v $t_{1/2}$, urine vol, UCr & CCr ($p<0.01$), weight & SA ($p<0.001$); UPARG+S v M.Sc & total UPAR ($p<0.01$); Total UPAR v M.Sc, urine vol & UPARG+S ($p<0.01$); SCr v CCr ($p<0.001$); CCr v Cl, weight & SA ($p<0.01$), UCr & SCr ($p<0.001$)

5.3.3 Male Subjects

Results & correlations from all male subjects are given in appendices F10 & F11. Males were well matched with respect to age & mobility score which were not correlated. Age & SCr were correlated ($p<0.01$), a relationship not seen with the females or the entire group. Overall, the male group

exhibited a similar pattern of correlations as the entire group, but the reduction in numbers caused most trends to only approach significance. Significant correlations were:- age v SCr ($p<0.01$); M.Sc v UPARG+S, total UPAR & UCr ($p<0.01$); Total UPAR & UPARG+S v M.Sc ($p<0.01$), UCr ($p<0.001$); UCr v M.Sc, UPARG+S, total UPAR, CCr, weight & SA ($p<0.01$); SCr v CCr ($p<0.01$); CCr v UCr & SCr ($p<0.01$).

5.3.4 Comparison of Male and Female Results

Results from females & males were compared using the Mann-Whitney test and the results are given in appendix F12. The pattern of correlations seen for females & males were similar with few exceptions. The males were better matched for age & mobility, with mobility scores evenly spanning the range of ages. Thus the correlation of age v mobility score seen for the female group was not reproduced for the males. The mean age was lower for the males (78±8y M, 82±8y F) but this difference was not significant. Mobility scores were similar.

The male group had a significantly greater $t_{1/2}$ (3.32±1.15h M, 2.80±0.69h F; $p<0.04$) but they exhibited a wider range and hence standard deviation. A significant difference also existed between [SPAR] at 3 & 4h post-dose reflecting the difference in $t_{1/2}$ between sexes.

SCr was significantly increased for the males (1.65±0.78 M, 1.20±0.50mg/dl F;$p<0.02$) likewise UCr (970±504mg M, 696±252 mg F;$p<0.05$).

The male group had a significantly greater weight (66±14kg M, 58±15kg F;$p<0.04$) & SA.

Cl, Cl/kg, urine vol, free UPAR, UPARG+S, total UPAR, CCr & CCr/SA were not significantly different between sexes.

5.4 COMPARISON OF PARAMETERS FOR FIT v FRAIL GROUPS

5.4.1 All Subjects

Subjects were divided into fit & frail groups according to the definitions given in appendix A1 and compared. Results for the entire group (females + males) are given in appendix F13 and Figs. 5.4.1 to 5.4.6.

There was a 6 year age difference between fit & frail groups (75±8y fit, 84±7y fr.;$p<0.01$), largely due to the lack of immobile females under 80y.

From the serum PAR data significant differences were seen between groups for $t_{1/2}$ (2.67±0.46h fit, 3.38±1.16 fr; $p<0.005$), Cl (18±7ml/min fit, 14±5ml/min fr;$p<0.02$) & Cl/kg ($p<0.05$). [SPAR] at 3h & 4h were not significantly different.

From the UPAR data no difference between fit & frail groups was found for free UPAR excretion, but UPARG+S & total UPAR were significantly greater for the fit group ($p<0.002$). This was reflected in the difference in excretion rate of total UPAR from 0-7 & 0-15h post-dose ($p<0.01$). Fit subjects passed significantly more urine during the 24h collection period (1392±551ml fit, 1037±465ml fr;$p<0.02$). SCr was similar for both groups (1.28±0.41mg/dl fit, 1.50± 0.85 mg/dl fr), while UCr & CCr were significantly greater for the fit group ($p<0.002$ & $p<0.05$). The difference in CCr/SA approached significance ($p<0.06$) but [UCr] did not. Weight & SA were comparable between groups.

5.4.2 Female Subjects

Fit & frail female groups were compared and the results are given in appendix F14. The females were poorly matched in terms of age, with an 8y difference between the mean age of the fit & frail groups (78±9y fit, 86±5y fr.;$p<0.01$).

The frail females had a significantly increased elimination $t_{1/2}$ (2.59±0.35h fit, 3.04±0.91h fr;$p<0.05$), although no statistical difference was seen between fit & frail females for Cl (17±8ml/min fit, 14±5ml/min fr), Cl/kg or [SPAR] at 3h or 4h.

While there was no difference between free UPAR excretion for the two groups, frail females excreted significantly less UPARG+S & total UPAR ($p<0.02$).

UCr excretion was significantly greater in the fit group (789±231mg fit, 556±221mg fr;$p<0.02$).

There was no difference in urine volume, [UCr], SCr, CCr or CCr/SA between the fit & frail groups who were matched in terms of bodyweight & SA.

5.4.3 Male Subjects

Fit & frail groups of male subjects were compared and the results are given in appendix F15. The two groups were well matched for age (76±8y fit, 80±9y fr).

From the serum PAR data $t_{1/2}$ was greater in the frail group (2.79±0.59h fit, 3.85±1.34h fr;$p<0.02$), who also showed a significant reduction in both Cl & Cl/kg (Cl: 21±5ml/min fit, 14±5ml/min fr;$p<0.01$). [SPAR] at 3 & 4h were also significantly lower for the fit males ($p<0.02$).

From the UPAR data there was no difference between the groups for free UPAR, but the frail males excreted significantly less UPARG+S ($p<0.02$) & total UPAR ($p<0.04$), with the rate of total UPAR excretion at 0-7h & 0-15h post-dose significantly lower in this group ($p<0.02$ & $p<0.005$).

Urine volume & UCr excretion were significantly lower in the frail group ($p<0.03$).

There was no difference between groups for [UCr], SCr, CCr, CCr/SA, weight & SA.

5.4.4 Age-Sex Matched Pairs

Some of the differences seen between the fit & frail groups could be confounded by their disparity in ages and so 32 subjects were age-sex matched to within one year to give 16 pairs. Results & correlations from these groups are given in appendices F16, F17 & F18.

With equalization of mean ages, the increased $t_{1/2}$ in the frail group remained (2.69±0.52h fit, 3.52±1.37h fr;$p<0.02$)

Cl & Cl/kg both tended to be reduced in the frail group (Cl: 17±5ml/min fit, 14±6ml/min fr) but neither reached statistical significance ($p<0.07$).

Free UPAR excretion was similar between groups while UPARG+S & total UPAR were again significantly reduced in the frail group.

Urine volume, UCr, SCr, CCr, CCr/SA, weight & SA did not differ significantly between fit & frail groups.

Fig. 5.4.1
Distribution of PARACETAMOL $t_{1/2}$ in Fit & Frail Elderly People

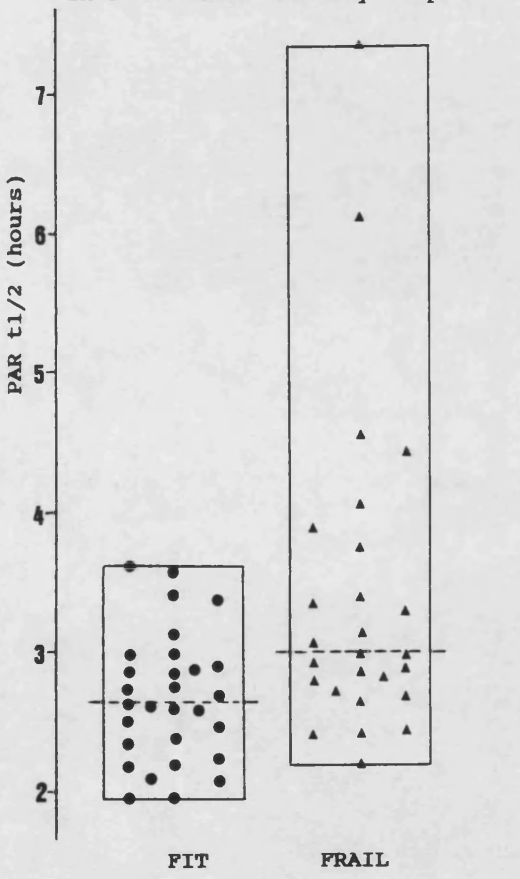


Fig. 5.4.2
Distribution of PARACETAMOL clearance in Fit & Frail Elderly People

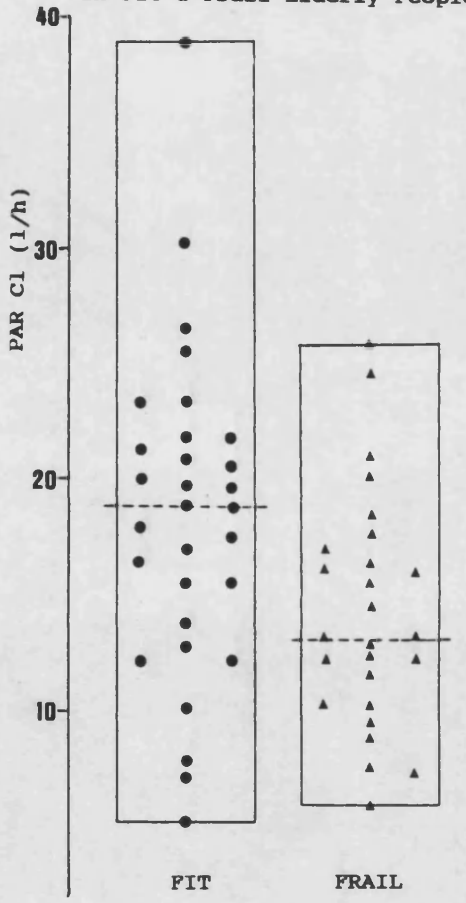
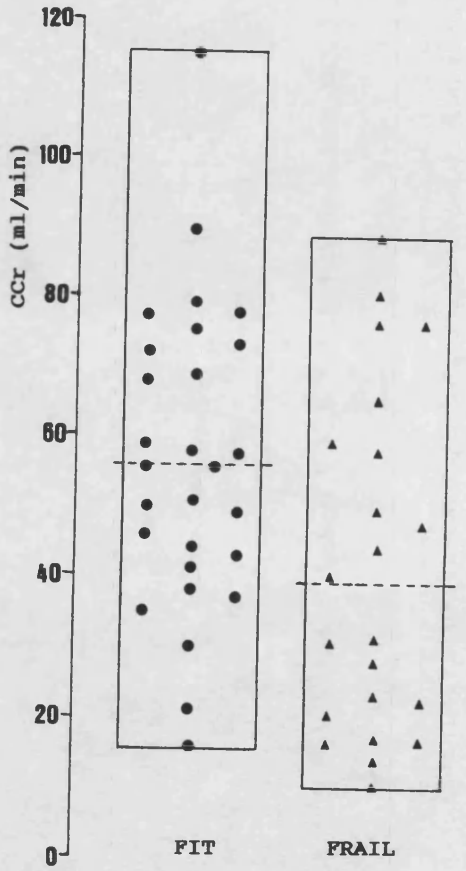


Fig. 5.4.3
Distribution of CCr in Fit & Frail Elderly People in the PARACETAMOL Study



● = fit subject

▲ = frail subject

---- median

Fig. 5.4.4
Distribution of free UPAR in
Fit & Frail Elderly People

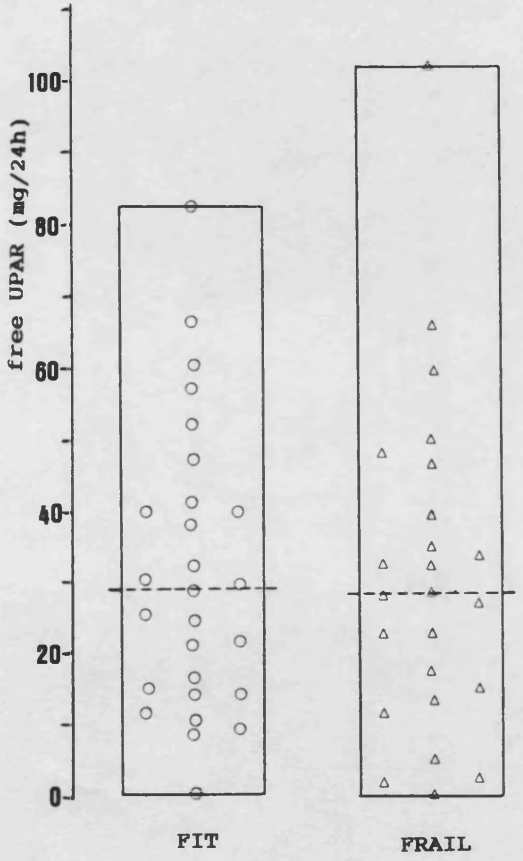


Fig. 5.4.5
Distribution of UPARG+S in
Fit & Frail Elderly People

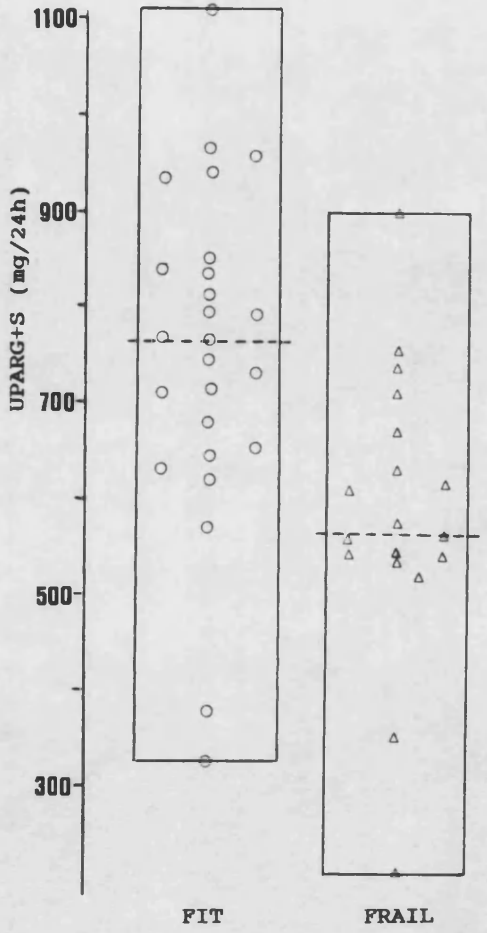
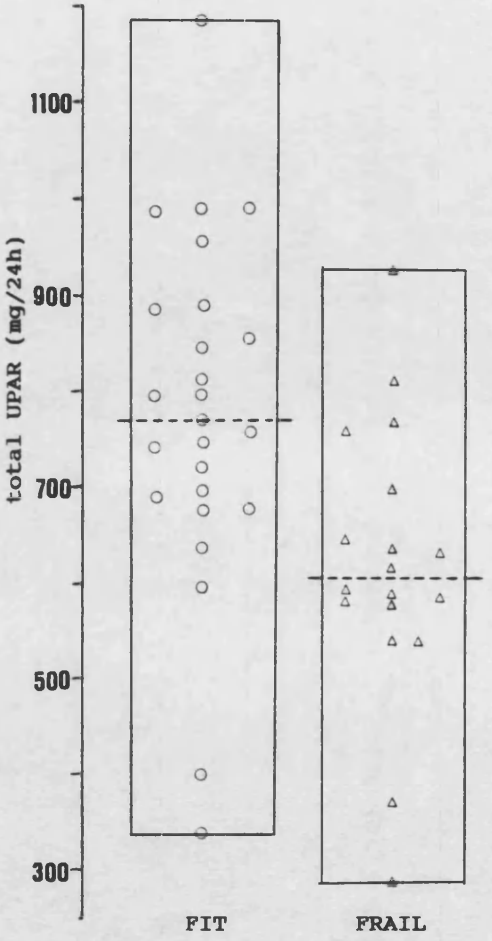


Fig. 5.4.6
Distribution of total UPAR in
Fit & Frail Elderly People



○ = fit subject

△ = frail subject

---- median

5.5 DISCUSSION

5.5.1 Introduction

In this study a therapeutic dose of PAR was given orally to 55 elderly people with varying degrees of immobility and frailty. Their mobility was scored from 1 (most mobile) to 5 using a rating scale devised for the study, according to their activity in general and during the study. Subjects were also categorized as fit or frail. The excretion of PAR was followed with serum PAR data allowing elimination $t_{1/2}$, AUC, apparent Cl & Cl/kg to be calculated. Urine PAR data enabled free UPAR, UPARG+S and total UPAR to be determined. SA, SCr, CCr and CCr/SA were also calculated.

5.5.2 Paracetamol Kinetics

29 fit (18F) & 26 frail (15F) elderly people took part in the study, with mobility scores ranging from 1 to 5. PAR kinetics from 3-12h after dosing were followed and appeared to undergo first-order kinetics, with log [SPAR] v time yielding a linear plot. This agrees with other studies of PAR pharmacokinetics (64). The sampling times chosen were past the peak [SPAR] and the maximal [SPAR] had been reached in every subject before the 4h sample. PAR absorption from a solid dose formulation is reported to give peak [SPAR] around 0.79h postdose in young volunteers (12) and 0.74h in elderly subjects (13), with distribution complete at 1-1.5h (212). Sampling times in the present study anticipated both the absorption and distribution phases to be well passed. The correlation coefficient of log [SPAR] v time approximated to unity in all cases ($r>0.947$), enabling accurate estimation of $t_{1/2}$, AUC & Cl.

There was a significant difference between the females &

males for $t_{1/2}$ and [SPAR] at 3 & 4h, and so for these the sexes will be discussed separately. No differences were seen for other pharmacokinetic parameters and so the sexes will be combined, and only the entire group discussed.

5.5.3 Elimination Half-life

A circadian variation in the rate of PAR elimination has been reported with $t_{1/2}$ being reduced by 15% when given at 6am compared to 2pm (213). PAR was therefore administered at a similar time of day for all subjects, between 0730 & 1050. The mean elimination $t_{1/2}$ for the female group was 2.80±0.69h (range 1.96-6.09h), and for males 3.32±1.15h (1.97-7.33h). The ranges, similar for both sexes, spanned an almost fourfold range. The mean $t_{1/2}$ are higher than those quoted in other texts but within the ranges cited. Reports of elimination $t_{1/2}$ have varied, ranging from 1.9-3.3h in young subjects (mean age 29y; 79) and 1.9-4.3h (79), 1.43-3.45h (80) & 1.9-4.3h (12) for elderly subjects with mean ages of 70, 77 & 71y respectively.

The group in the present study were older (80.3±8.3y) than elderly groups in other studies, who have used only healthy volunteers. The markedly raised $t_{1/2}$ seen here in a few individuals suggests that the physical condition of the subject may influence the rate of PAR elimination.

Elimination $t_{1/2}$ was not related to age for either sex, even when subjects were grouped into ten year cohorts, and the mean $t_{1/2}$ for each group compared. This study aimed to determine changes in PAR kinetics with mobility and frailty in old age, so the age range examined was narrower than in other studies where young and elderly groups have been compared. Comparisons of PAR $t_{1/2}$ in young and elderly volunteers are inconclusive, with different authors stating

both that age increases PAR $t_{1/2}$ (80,13) and does not affect PAR $t_{1/2}$ (79).

The female group had a significantly lower $t_{1/2}$ ($p<0.04$) and [SPAR] at 3 & 4h than the male group, while Cl & Cl/kg were not statistically different. The males were on average 8kg (14%) heavier than the females with a 12% greater SA. Drug distribution is known to be influenced by body composition & weight, and PAR has been shown to distribute into excess bodyweight over ideal bodyweight by a factor of 0.44 (108). This suggests an incomplete distribution of the non-lipophilic drug into body fat. References report a decline in PAR Vd with age, when a higher proportion of bodyweight comprises of fat, with elderly females having a lower Vd than elderly males (79, 80). Since $Cl = (Vd \times 0.693)/t_{1/2}$ it is possible that a reduction in both Vd & $t_{1/2}$ could produce no change in Cl. It therefore seems likely that inequality in weight & body composition between sexes may be an important factor in the explanation of the reduced $t_{1/2}$ seen in the female group. Furthermore, the amount of UCr excretion was significantly greater in the male group although [UCr] was comparable, emphasizing the difference in body composition (especially muscle mass) between the sexes.

Elimination $t_{1/2}$ is influenced by both Cl & Vd, with Vd being related to body weight and composition. Cl, which is independent of Vd is therefore a preferable pharmacokinetic parameter to use when comparisons are being carried out, particularly when weight & SA are not closely matched between groups (9). Sex differences have not been noted by other groups (12,79,80) and since Cl & Cl/kg were not statistically different for males & females, the disparity

in $t_{1/2}$ would appear to be for the above reasons. Elimination $t_{1/2}$ was correlated with Cl & Cl/kg, and since $t_{1/2}$ is mathematically related to Cl this correlation is to be expected. However, $t_{1/2}$ was not correlated with other parameters measured.

5.5.4 Apparent Clearance

In order to calculate apparent Cl it was assumed that PAR absorption was complete and did not differ between groups. PAR is known to be readily absorbed from the small intestine, and is thought to undergo first-pass metabolism in the liver, with a bioavailability of about 90% for a 1g dose (146). From the data collected it could not be shown unequivocally that bioavailability was comparable for all subjects studied and so the apparent (relative) clearance was calculated, using the dose administered rather than that reaching the circulation.

From the literature neither age nor renal impairment appears to significantly influence the extent of PAR absorption although the rate may vary (9,12,13,74,212). PAR is absorbed from the small intestine and absorption rate is therefore related to the rate of gastric emptying (64). Absorption is slowed if gastric emptying is delayed by food, posture or certain drugs, and increased by drugs such as metoclopramide, but the total amount absorbed is not affected.

In the present study factors known to affect the rate of PAR absorption were standardized in order to maximize absorption without disruption to the volunteers normal routine. All subjects were allowed only a light breakfast at least one hour before the start of the study, and abstained from further food for at least an hour after PAR

administration. Tablets were administered either with the patient sitting upright or standing, although posture during the study varied. Only subjects F26 & M35 were taking drugs known to affect gastric emptying (both oral S/R morphine sulphate, appendix F3).

Previous studies have shown PAR Cl, when administered orally or iv, to be reduced in old age, but the magnitude with which Cl has varied with age has been inconclusive (13,74,79,80,81). In the present study mean Cl for the entire group was 16.3±6.4l/h (range 5.3-38.7), and Cl/kg 0.27-0.10l/h/kg (0.12-0.59). These values are similar to those reported in other studies where oral PAR was administered to elderly people. A mean Cl of 0.25±0.08l/h/kg was calculated using data from 28 healthy elderly volunteers with a mean age of 77 years (80). Individual Cl values were highly variable spanning a sevenfold range and Cl/kg an almost fivefold range.

The excretion of PAR conjugates would be expected to be positively linked to the rate of PAR elimination, since PAR glucuronide and sulphate form the majority of metabolised PAR. Cl correlated with CCr & CCr/SA, but not with SCr. Although unchanged PAR is hepatically metabolised, its metabolites are renally excreted. Therefore if Cl is related to conjugate formation, and their rate of excretion is related to renal function, then a correlation between PAR Cl & renal function is likely to result. Since SCr is a less satisfactory measure of renal function than CCr, particularly in cases of muscle wasting, this may explain why SCr does not appear to be correlated with Cl while CCr is. Cl/kg was not correlated with any of the parameters

suggesting that the correlations for Cl were at least in part due the way in which weight was related to the other parameters.

5.5.5 Free Paracetamol in Urine

Between 0-10% (31±21mg) of the dose administered appeared in urine as free PAR. This agrees with other reports where it is cited that unchanged PAR forms approximately 2-5% of PAR excreted in the urine (64). Free UPAR was not affected by any other parameter examined. Excretion of free UPAR is via glomerular filtration although subsequent extensive passive tubular reabsorption occurs (212), thought to be primarily dependent on urine flow rate. These results suggest that passive tubular reabsorption is not disproportionately affected by age or mobility.

5.5.6 Paracetamol Glucuronide & Sulphate Excretion in Urine

Recovery of total UPAR averaged 715±185mg with PAR glucuronide & sulphate forming between 118-1110mg (681±185; 68%) of the total. As a proportion of all PAR excreted, approximately 55% is in the form of UPARG & 30% as UPARS (214). Results from the present study are within this range.

UPARG+S elimination was correlated with mobility score & CCr. Polar conjugates are excreted primarily by active tubular secretion and so it would be expected that their elimination is strongly influenced by CCr as the results show. This agrees with others examining PAR dispositon in patients with renal failure (212). Their study showed that while excretion of free UPAR differed little between normal subjects and patients with renal failure, elimination of PAR conjugates was increasingly impaired with a decline in

renal function.
CCr is documented as declining in old age (123) and this correlation is seen in this study. This most likely explains the correlation of UPARG+S & age. The correlation of mobility score with CCr may explain the relationship between UPARG+S excretion & mobility score but it is possible that conjugate excretion is affected by mobility.

5.5.7 Total Urinary Paracetamol

Correlations for total UPAR are similar to those for UPARG+S, since total UPAR = free UPAR + UPARG+S. UPAR conjugates form a greater proportion of total UPAR excreted than free UPAR, so the similarity in correlations is to be expected.

5.6 PARACETAMOL KINETICS IN FIT & FRAIL ELDERLY PEOPLE

5.6.1 Sex Difference

Fit & frail groups were further divided into male & female groups, and trends seen were similar between them. Therefore, fit & frail groups are of mixed sex except where specific differences were seen - these are discussed under each heading where applicable.

5.6.2 Comparison of Age

Results from 29 fit & 26 frail elderly were used in this study. Fit subjects were on average 6y younger than their frail counterparts (77$\pm$8y fit, 84$\pm$7y fr.;$p<0.01$), largely due to the scarcity of frail females below the age of 80y. Fit & frail males were matched with respect to age ($p<0.3$). 32 subjects were age-sex matched to enable the influence of age on parameters to be excluded. This is discussed in 5.6.9.

5.6.3 Mobility Scores

The two groups differed significantly in their mean mobility scores as would be expected, since the mobility of the subject was taken into consideration when they were categorized as fit or frail. Fit subjects had a mean mobility score of 1.5±0.7 & frail subjects a mean score of 3.7±0.7. There was some overlap between groups with respect to mobility score, with 3 fit and 11 frail people having a score of 3. This is due to the definitions used taking into account not only mobility but also clinical condition of the subject. The remaining fit elderly scored 1 (n=18) or 2 (n=8), and the frail elderly 4 (n=11) or 5 (n=4).

5.6.4 Comparison of Elimination Half-lifes

Mean PAR elimination $t_{1/2}$ was 2.67±0.46h (1.96-3.60) for the fit group compared to 3.38±1.16h (2.20-7.33) for the frail group, and this difference was highly significant ($p<0.003$). While there was some overlap between groups, a distinct difference could be seen, with a wider variation in $t_{1/2}$ for the frail group (Fig. 5.4.1).

Elimination $t_{1/2}$ is affected by Vd & Cl, and it is possible that the difference could be explained by changes in Cl or Vd occurring with increasing frailty. Weight & SA were similar between groups suggesting that Vd was also comparable. It is worthy of note that the significant difference in $t_{1/2}$ remained when groups were further divided into females & males or age-sex matched pairs, suggesting that a difference in Vd is unlikely to be responsible for this result.

5.6.5 Comparison of Apparent Clearance

The true Cl could not be calculated since PAR was given

orally necessitating instead the calculation of apparent Cl. While fit & frail groups were matched for weight & SA, a significant difference was observed between mean Cl ($p<0.02$), with the difference in Cl/kg approaching significance ($p<0.06$). The frail group showed an overall reduction in Cl & Cl/kg which approximated to 77% & 83% of those of the fit group, although a degree of overlap was seen (Fig. 5.4.2). This suggests that frailty may be an important influence on PAR clearance, but not the sole determinant. On considering the entire group the effect of age could not be excluded, due to an age difference of 6y between groups. For this reason groups were subdivided to give two smaller age-sex matched groups and the results are discussed in 5.6.9.

The influence of diet on conjugation reactions in man has been examined (215). It has been found that PAR Cl undergos a 17% increase following a diet high in cruciferous vegetables for 10 days, with an 8% increase in recovery of PARG in urine 24h post-dose. This may explain the differences observed since individuals in the community may well consume a diet different both in content and calories than those in institutions.

5.6.6 Comparison of Free Paracetamol in Urine

The absolute amounts of free UPAR did not differ between fit and frail groups, with the ranges and mean values being comparable (Fig. 5.4.3). No correlations were seen between mobility, age, Cl or CCr and free UPAR and this finding would suggest that frailty does not influence free UPAR excretion either. Unchanged UPAR is eliminated by glomerular filtration with extensive passive reabsorption,

and these findings suggest that this method of elimination does not exhibit a reduction in capacity with frailty.

5.6.7 Paracetamol Glucuronide & Sulphate Conjugate in Urine

The amount of conjugated PAR excreted was significantly reduced in the frail elderly, with the mean UPARG+S approximating to 80% of that for the fit elderly. Again there was overlap between the ranges for the groups (323-1109mg fit, 188-896mg fr.) but the frail group occupied a distinctly lower range (Fig. 5.4.5).

This implies that either less conjugate was formed, leading to the reduction in Cl & $t_{1/2}$ observed in the frail group, or else the conjugates were formed at a similar rate but not as efficiently excreted. In the latter case conjugates could accumulate particularly if active tubular excretion was reduced in frailty. This cannot be concluded from this study, since the passage of the conjugates in blood was not followed. CCr was significantly different between groups, with the frail group showing an overall reduction in CCr ($p<0.04$). This again would suggest that the conjugates could accumulate, particularly if the patient was on long-term, regular therapy.

When the sexes are separately considered, CCr is comparable for fit & frail groups, while the amount of PARG+S excreted remained significantly less in the frail groups. This would indicate that less conjugate was formed, rather than it accumulating due to a reduced CCr.

5.6.8 Comparison of Total Paracetamol Excreted in Urine

There was a significant difference between the absolute quantities of free plus conjugated UPAR (total UPAR) excreted in the urine, with the frail group excreting on

average 80% of that excreted by the fit group (782±184mg fit, 623±148mg fr; $p<0.002$). This can be explained by observing excretion of free UPAR & UPARG+S. No difference in free UPAR excretion was seen between fit & frail groups, while the elimination of UPARG+S differed significantly. The sum of these components gives total UPAR, and PAR conjugate in urine exceeds that of the free PAR. The contribution of UPARG+S is therefore greater, leading to the observed significance, reduced compared with UPARG+S alone.

The rate of total UPAR excretion from 0-7h & 0-15h postdose was found to be significantly lower for the frail group. From these results it is not possible to determine whether a reduction in the formation of conjugates or their accumulation is responsible for their reduced elimination rate.

It would seem likely, given the differences in $t_{1/2}$ & Cl between the fit & frail groups, that formation of conjugates is reduced in frailty. From this study it is not possible to determine whether glucuronide or sulphate formation, or both, are reduced. The lower CCr seen in the frail group suggest that PAR conjugates could accumulate in the body, contributing to the reduced recovery in frailty. The result is that PAR is cleared less efficiently in the frail person, with sustained levels of both PAR and its conjugates, although the effects of increased levels of PAR and PAR conjugates are not known.

5.6.9 Comparisons Between Age-Sex Matched Groups.

Because the differences observed between fit & frail groups could be exaggerated by the difference in mean ages, a

subgroup was formed using an age-sex matching technique. Pairs of individuals of the same sex were formed having a similar age ± 1 year. Mobility scores between individuals in a pair were as diverse as possible. 16 age-sex matched pairs resulted who formed fit & frail groups. Differences between these groups were again examined (appendix F18).

Mean elimination $t_{1/2}$ remained significantly shorter in the fit group despite the reduction in numbers ($p<0.03$), and the range of values was narrower in the fit group (1.97 - 3.60h) than the frail group (2.20-7.33h). Both Cl & Cl/kg were lower in the frail group but this trend failed to reach significance ($p<0.07$). Free UPAR excretion was unchanged between groups, but UPARG+S & total UPAR excretion were reduced significantly in the frail group, who also had a significantly lower rate of total UPAR elimination over 0-15h postdose ($p<0.03$). CCr, SCr, urine volume, UCr, weight & SA were similar between groups.

5.6.10 Relevance of Tobacco and Alcohol Consumption

6 fit & 3 frail subjects smoked tobacco, although only one from each group smoked in excess of 10 cigarettes per day. Both $t_{1/2}$ & Cl values for smokers were distributed evenly throughout the ranges, suggesting that smoking was not likely to be responsible for the differences observed although the numbers involved are too small for a definitive statement. Moderate smoking (about 10/day) has been reported as having little effect on PAR metabolism, while heavy smoking (about 40/day) appears to induce conjugation with glucuronic acid, but not sulphates (163).

1 fit & 2 frail subjects admitted to excess consumption of alcohol (>14 units/week F, >21 units/week M). With only 3

participants known to drink alcohol in excess the importance of this cannot be determined from this study.

5.7 CONCLUSIONS

In this study 1g of PAR was given to 55 elderly people with varying degrees of immobility & frailty. PAR in serum was followed from 3-12h post-dose, and a 24h urine collection was carried out. Various pharmacokinetic parameters were calculated from the serum data, and PAR & PAR conjugate excretion in urine was measured.

Mobility score appeared to be associated with an increase in $t_{1/2}$ and a reduction in Cl, although these correlations were only of borderline significance. However, an increase in mobility score was strongly correlated with a reduction in excretion of both UPARG+S & total UPAR, while the elimination of free UPAR did not change significantly. Frailty was consistently associated with an increase in $t_{1/2}$ and reduction in Cl & Cl/kg; the change in $t_{1/2}$ remained significant when individual sexes were considered, while the difference in Cl approached significance. In all cases free UPAR did not appear to be affected by frailty while UPARG+S & total UPAR were significantly reduced. These observations could be the result of a number of differences between fit & frail elderly.

A difference in absorption of PAR seems to be an unlikely explanation for the differences between the fit & frail groups. Authors have found that absorption rate is affected by factors such as age, posture, gastric emptying rate & diet, but there are no reports of an age-related reduction in the extent of drug absorption via passive

diffusion (9,12,13,74,212,214). Although increased PAR absorption with frailty could explain the elevated [SPAR] at 3 & 4h, the increased $t_{1/2}$ & reduced Cl could not be accounted for by a change in absorption since PAR kinetics are independent of dose. Furthermore, the reduced recovery of total UPAR also indicates that increased PAR absorption in the frail group is unlikely.

Dietary intake could differ between those who are frail & institutionalized and those who are fit & self-caring in the community. PAR metabolism is known to be increased in subjects eating a diet high in cruciferous vegetables, when it seems that induction of the glucuronidation pathway by certain indoles occurs (215). Diets consumed by the fit & frail groups were not standardized, but many elderly people, even those who are relatively mobile, often appear to consume a diet lacking in fresh vegetables for economic reasons (49).

Each subject took the PAR tablets in the upright position, either sitting or standing. This was the only attempt made to standardize posture during the study. The frail group were much less mobile than the fit group, and tended to spend longer in bed and more time semi-recumbent or supine. It has been noted that bed rest per se can bring about pronounced changes in distribution, metabolism & excretion of drugs relative to values obtained in ambulatory subjects (209). One study followed serum levels & urinary excretion of i.m. benzylpenicillin in subjects when in bed and subsequently when ambulatory (41). The fraction of the dose excreted unchanged and the renal clearance of the drug were significantly increased, and the first-order rate constant

was significantly decreased during bed rest. This was thought to be due to an increased "metabolic rate" in ambulatory subjects or marked plasma circulatory volume differences found in upright and recumbent subjects (208).

It is possible that PAR metabolism may also be influenced by the above factors. However, prolonged bed rest can cause a deterioration in cardiovascular function which is associated with a decrease in plasma volume, and many of the frail subjects had been immobile for some time. It is not possible to say whether a difference in posture could at least in part account for the changes in PAR metabolism seen in frail subjects, but it can certainly not be discounted.

Associated with the differences in posture seen between the two groups of elderly subjects is the obvious variation in physical activity. The fit group were generally more active than the frail group who had a very restricted pattern of activity. Studies have investigated the effect of both acute & chronic exercise on drug disposition. One examined the effect of chronic exercise in a group of young people using each subject as their own control (216). It was found that as fitness improved, as measured by an increase in maximal oxygen uptake, so the hepatic metabolism of model drugs antipyrine & aminopyrine also increased. It is not known whether the enhanced hepatic metabolism was accompanied by changes in other liver enzymes. This result has been reproduced in other studies suggesting that chronic exercise which increases physical fitness is accompanied by accelerated hepatic biotransformation of low clearance drugs.

Studies on the effect of chronic exercise on the metabolism of high clearance drugs however, have been inconclusive (216).

The effect of acute physical exercise on liver blood flow has been studied using indocyanine green (ICG) which has a high hepatic extraction ratio. ICG $t_{1/2}$ is prolonged by vigorous physical exercise and the greater the degree of exercise the greater the prolongation in $t_{1/2}$ (217). Using this method a 60% reduction in liver blood flow during exercise has been estimated. A second acute exercise study involved the use of propranolol, another high clearance drug (218). During exercise ICG Cl was significantly reduced but propranolol exhibited a reduced $t_{1/2}$ & AUC. This result was unexpected since the ICG Cl indicated a reduction in liver blood flow, and so a rise in propranolol $t_{1/2}$ was also anticipated. The explanation offered for this was that the absorption of the drug may have been affected, although other studies have not shown this to be likely. The reduction in liver blood flow seen with vigorous exercise could have resulted in a lower bioavailability of propranolol, explaining the reduced AUC. Exercise could have also increased the Cl of the drug but this seems to be contrary to a reduced liver blood flow. The study concluded that other factors besides exercise may have influenced propranolol Cl on the study day. Low clearance drugs, such as antipyrine, with capacity limited hepatic elimination do not appear to undergo a change in metabolism with acute exercise.

No studies have been carried out on the effect of acute or chronic exercise on the kinetics of PAR, a low clearance drug exhibiting capacity limited metabolism. Studies on

the metabolic effects of chronic exercise are more likely to be relevant to this study than those on acute exercise. The frail elderly were not as physically fit or active as their fit counterparts. Since metabolism of other drugs with a low hepatic extraction ratio appears to be stimulated by an increase in physical activity, it is possible that the same could be true for PAR. Although the actual activity of each subject was not studied the mobility scale and the fit & frail definitions included an assessment of each subjects activity. It would seem likely that the increased activity seen in the fit subjects may increase their general "metabolic rate" and stimulate liver blood flow thereby enhancing PAR metabolism.

During hepatic senescence liver histology appears to alter little. There is a rise in the average cell size in people over 60 years and an increase in the number of cells with large nuclei, but the significance of this is not clear (30). It is now well documented that both liver size and blood flow decline with age as discussed in 1.1 (9,21,30, 81,219,220).

In the present study age & $t_{1/2}$ were not correlated while a negative correlation was found between age & Cl, even over the small range of ages studied. When fit & frail groups were compared $t_{1/2}$ & Cl were significantly different, suggesting that other factors besides age difference were important in the determination of PAR metabolism. When subjects were subdivided to give two age-sex matched groups, the significant difference between $t_{1/2}$ remained while the difference in Cl approached significance.

This agrees with another study where PAR Cl was found to be greatest in young people compared with healthy elderly

people, with those elderly people who were frail having the lowest PAR Cl (81). When PAR Cl was expressed per unit volume of liver, no difference was found between fit young & elderly people but there was a significant difference between the fit & frail elderly.
While we were not able to measure liver volume in the present study, it would seem likely that a similar case would be found if we had. That is, that the reduction in liver volume in old age is partly responsible for the decline in Cl seen for many drugs with increasing age, but an additional factor, such as the general health of the subject, adds an additional decrement to the hepatic clearance of a drug.

It is possible that the frail elderly may have reduced liver perfusion resulting in increased drug bioavailability. Following first-pass metabolism approximately 90% of an oral dose of PAR is found in the circulation. Any changes in bioavailability would have to be extreme before changes were seen in the order of this study, although any changes occurring as a result of frailty may be more important for drugs with a much lower bioavailability.
It is possible that the enzyme systems responsible for conjugation reactions have a lower activity or affinity for substrate, to explain the results observed. A reduction in conjugation would produce sustained [SPAR] levels and less conjugate excretion into urine, as this study found. From the literature it would appear that enzyme systems may be variably affected by age & frailty. Frailty rather than age has been shown to influence plasma levels of the enzyme aspirin esterase, found to be significantly lower in a

group of frail hospitalized elderly patients compared with healthy elderly & young volunteers (221). Conversely an in vitro study failed to detect an age associated decline in microsomal mono-oxygenase activity or affinity in man (222). The effect of frailty on this system was not been determined in vitro.

Acetanilide Cl has been found to be similar in fit & frail elderly but significantly lower than in young subjects. However, when Cl was expressed per unit volume of liver no difference in Cl was found, suggesting that a decline in liver size was responsible for the reduced Cl seen in old age (220).

The present study only compared PAR Cl in fit & frail elderly people and so a definitive statement on the importance of age cannot be made. It would appear that frailty does have an effect on PAR metabolism, and it is possible that liver volume and/or blood flow could be reduced in the frail elderly compared to their fit counterparts.

Glucuronide & sulphate conjugates were not assayed separately in urine or in serum and so the influence of frailty upon conjugate formation cannot be commented on. Frail subjects excreted significantly less UPARG+S & total UPAR, suggesting that their formation may be impaired. It is possible that the reduction in CCr seen in the entire group when comparing fit & frail subjects was responsible for this. When CCr was comparable, for single sex & age-sex matched groups, the increase in $t_{1/2}$ and reduction in Cl remained. As previously discussed, patients with renal impairment have been found to excrete less PAR conjugate although it appears to be formed at a similar rate than

those with normal kidney function, leading to accumulation of conjugate in the body (212). In a study where PAR conjugates were quantified, glucuronide, but not sulphate conjugate formation was impaired in frail subjects compared to fit subjects of a similar age (81). This could offer an explanation to the results observed in the present study, since a sustained [SPAR], increased $t_{1/2}$, reduced clearance and recovery of UPARG+S would fit with this hypothesis.

In summary, PAR elimination $t_{1/2}$ was increased and apparent clearance reduced in subjects who were frail & immobile compared with their fit, mobile counterparts of similar age. Various hypotheses could explain these findings.
It is possible that PAR absorption was altered in the frail group but from the literature this seems unlikely.
Diet may have varied between fit & frail groups, and it is known that certain foods, and chemicals contained in them, can induce metabolic pathways. A diet containing more fresh vegetables consumed by the fit group could explain the findings.
A difference in activity & posture between the two populations could also afford an explanation, with differences in plasma & circulatory volumes, and changes in liver perfusion, causing the inequality in PAR metabolism observed.
Liver size & blood flow are known to be reduced in old age, and they may be reduced further in frailty. Any reduction in liver volume could reduce the metabolism of a hepatically cleared drug with a low extraction ratio.
Enzymes responsible for conjugation reactions in the liver could have a reduced activity or affinity in frailty explaining both the reduction in elimination and the lower

recovery of PAR conjugates in urine.

Although none of these explanations can be proven from the present study, it is highly probable that more than one factor is important. Changes in activity & posture may well be important, perhaps in some way influencing the activity of the conjugating enzymes, which appear to reduce PAR conjugation reactions.

The effect of sustained PAR levels in the body is not known, nor the accumulation of PAR conjugates which is likely to occur when renal function is reduced. However, it would seem reasonable that PAR is prescribed with caution in the very old, frail elderly person.

Further work needs to be carried out to determine whether a similar effect is seen for other drugs eliminated by conjugation reactions.

CHAPTER SIX

DIGOXIN EXCRETION IN ELDERLY PEOPLE

6.1 INTRODUCTION

Digoxin has been used in therapeutics for over two hundred years but its use is still associated with increased mortality & morbidity. Digoxin has a narrow therapeutic index (0.8-2.0ng/ml) and it is desirable to know which factors most influence the rate of elimination in order to prevent toxicity. For this reason digoxin (DIG) disposition has been extensively researched, with studies using both healthy volunteers & patients. While many studies have used elderly subjects, none have compared DIG excretion in those with varying degrees of immobility & ill health.
In this study DIG excretion was investigated in elderly people who were either "fit" (mobile, controlled chronic illness, living independently) or "frail" (immobile, poorly controlled chronic illnesses, loss of independence) in an attempt to determine whether frailty & immobility affected the efficiency of DIG elimination.

6.2 PROCEDURE

Fit & frail elderly people aged over 64y were invited to take part in the study. All were chronically ill, mostly with AF or CCF, and had been regularly taking the same dose of DIG for a minimum of 3 weeks. Exclusion criteria are given in 2.2. Each subject was given an information sheet (appendix E1, standard or enlarged type) and had the opportunity to question myself or Dr L Parker (GP trainee) about the study prior to its commencement.
On the morning of the study subjects emptied their bladder

and took their usual DIG tablet with a glass of water, in the upright position. DIG dose varied according to individual prescriptions which were not altered for the study. All tablets were manufactured by Wellcome (Lanoxin), but were from different batches as dispensed by the patients' own Pharmacist. Tablets were generally taken after a light breakfast of toast or cereal, with food and drink being freely taken thereafter.

During the study subjects were questioned about their day-to-day activity & mobility, categorized as fit or frail and had their mobility scored according to the mobility rating used in the study (appendix A1, A2). The form given in appendix E2 was completed during the study when subjects were weighed & measured and had their co-medication noted. Demographic details & drugs are given in appendix E3.

The ideal sampling time for DIG has been calculated to be 11h post-dose, when [SDIG] best estimates DIG concentration at "steady-state" (114). 5-10ml of blood was therefore taken at 11 & 24h and urine collected for 24h after dosing. DIG was assayed in serum at 11h & all urine samples, UCr in all urine samples, SCr in serum at 24h. Urine collected over 24h was assayed in 3 aliquots each as near to 8h as possible, the final aliquot spanning the hours spent in bed overnight. Analytical methods are given in chapter 2. About 5 days later a second blood sample was taken 11h post-dosing. [SDIG] on the two occasions were compared to ensure that subjects were in steady state ([SDIG] samples did not differ by > 15%), and the mean value used in subsequent calculations.

Total body clearance & renal clearance were calculated and expressed per kg bodyweight & 1.73m^2 SA as below:-

$$\text{Total body clearance Clt (ml/min)} = \frac{\text{dose x fraction absorbed}}{\text{[SDIG] x dosing interval}}$$

where fraction absorbed was 67%

$$\text{Renal clearance Clr (ml/min)} = \frac{\text{DIG recovered in urine/24h}}{\text{[SDIG]}}$$

$$\text{Non-renal clearance Clnr (ml/min)} = \text{Clt - Clr}$$

UCr, SCr & CCr were also determined for each subject.

Compliance

On initiation of therapy, steady state is only reached when 5 half-lives have elapsed, that is for DIG about 8 days in healthy subjects, if a set dosage is administered each day. If a loading dose is given, steady state is reached sooner (44). The therapeutic range is only valid if steady state has been reached and sampling is at least 6h post-dose when absorption & distribution are complete (114). If compliance is poor the relationship between dose, [SDIG] & therapeutic range does not hold, and a low [SDIG] could be indicative of underdigitalisation or failure to comply. All participants when asked "how frequently do you take your heart tablet?", gave the reply "every day", but compliance of all subjects could not be assumed. This is a continual problem when steady state kinetics are investigated, particularly when ambulant subjects are used who take medications unsupervised.

49 subjects (26F) successfully completed the study of whom 25 were frail. Results were considered statistically for the entire group (F + M) and for separate sexes.

Correlations were determined using Spearmans Correlation Coefficient and significance levels taken to occur when $p<0.01$. Fit & frail groups, and males & females, were compared using the Mann-Whitney U test with significance taken to occur at $p<0.05$.

6.3 RESULTS OF PHARMACOKINETIC PARAMETERS

6.3.1 Female Subjects Taking Digoxin

Results from individuals are given in Appendices E4 & E5, with results & correlations from the female group summarized in appendices E6 & E7.

Age ranged from 73-96y (82±6y; mean±s.d.) and correlated with no other parameter studied. Mobility score ranged from 1-5 (2.8±1.4), correlating with Clt & Clt/kg ($p<0.01$). DIG dose was 0.0625, 0.125 or 0.25mg daily (0.135±0.07) and correlated with [SDIG] ($p<0.01$) & UDIG ($p<0.001$).

Urine volume/24h varied from 490-2059ml (1133±461), while DIG in urine in 24h, UDIG, varied from 19.4-165.9ug (57±33) correlating with only with DIG dose ($p<0.001$). Percent dose recovered in urine in 24h, %Du, varied from 19.0-82.8% (45±19) correlating with Clr, Clr/kg, Clnr & Clnr/kg.

Serum DIG concentration [SDIG], ranged from 0.14-3.00ng/ml (0.91±0.72) and correlated with dose ($p<0.01$); Clt, Clt/kg, Clr & Clr/kg ($p<0.001$).

Total body clearance Clt, varied from 19-224ml/min (95±54) for females, & Clt/kg from 0.40-4.47ml/min/kg and both correlated with MSc, SCr & CCr ($p<0.01$); [SDIG], Clr, Clr/kg & Clnr ($p<0.001$).

Renal clearance Clr, varied from 10-171ml/min (66±47) and Clr/kg from 0.18-3.30ml/min/kg (1.23±0.88). Both correlated with SCr, CCr & %Du ($p<0.01$); [SDIG], Clt & SCr.

SCr ranged from 0.34-2.69mg/dl (1.09±0.66) correlating with CCr, Clt, Clt/kg, Clr & Clr/kg ($p<0.01$). CCr varied from 9-141ml/min (63±32), and CCr/SA from 11-167ml/min/1.73m^2 (70±38). CCr correlated with SCr, Clt, Clt/kg & Clr/kg ($p<0.01$) & Clr ($p<0.001$).
Weight varied from 35-76kg (53.9±10.1) and did not correlate with any parameter measured.

6.3.2 Male Subjects Taking Digoxin

23 subjects studied were male and results & correlations from this group given in appendices E8 & E9. Males were less old than females - male age varied from 66-89y (78±6) while mobility score varied from 1-4. Age correlated with dose & UDIG ($p<0.01$). Mobility score correlated with CCr ($p<0.01$). Correlations of age v Clt & Clr seen in the female tended towards significance for the males.
DIG dose was 0.0625, 0.125 or 0.25mg daily (0.152±0.07) and correlated with UDIG ($p<0.001$) as for the females, in addition with age & Clt. Urine volume/24h was greater for males than females, varying from 492-3742ml (1603±784). UDIG & [UDIG] were similar for both sexes, UDIG varying from 23-164ug (69±34) and correlating with dose and also age, CCr & Clr. %Du was also comparable, ranging from 25.0-84.9% (47.7±16.4) for males correlating with Clnr & Clnr/kg ($p<0.001$).
[SDIG] ranged from 0.26-1.70ng/ml (0.65±0.36), correlating with Clt/kg & Clr/kg ($p<0.01$) as for the females.
Clt ranged from 34-264ml/min (128±69) & Clt/kg from 0.55-3.46ml/min/kg (1.58±0.9), correlating with dose, CCr, [SDIG], Clnr & Clnr/kg ($p<0.01$); Clr & Clr/kg ($p<0.001$). These relationships are similar to those seen for the female group.

Clr varied from 27-194ml/min (85±45) and Clr/kg from 0.42-2.28ml/min/kg (1.17±0.55), correlating with UDIG & [SDIG] ($p<0.01$); CCr, Clt & Clt/kg ($p<0.001$), in a pattern similar to that for the females.
SCr ranged from 0.55-2.37mg/dl (1.13±0.42) and correlated with no other parameter. CCr ranged from 29-193ml/min (82±33) and CCr/SA from 37-164ml/min/1.73m^2, correlating with MSc, UDIG & Clt ($p<0.01$) and Clr ($p<0.001$).
Weight varied from 41-96kg (71.6±12.4) for the male group.

6.3.3 Comparison of Female & Male Results

Results from female & male groups were compared using the Mann-Whitney Test and results are given in appendix E10. Males were younger than females (78±6y M, 82±6y F) and this difference approached significance, but the groups had similar mobility scores. DIG dose prescribed was similar for male & female groups, with UDIG, %Du & [SDIG] being comparable between groups, although urine volume was greater in the male group (1603±784 M, 1133±461 F;$p<0.02$). UCr excretion was also significantly greater for the male group (1273±484mg M, 816±475mg F;$p<0.001$), with the difference in [UCr] tending towards significance between groups. SCr was similar between groups (1.13±0.42 M, 1.09±0.66 F) but CCr was greater for the males (82±33 M, 63±32 F $p<0.03$) while CCr/SA was not significantly different.
Clt, Clr & Clnr were not significantly different between sexes even when expressed per kg or 1.73m^2 SA.
These results suggest that the male & female groups studied did not differ in their ability to eliminate DIG. Results were therefore combined and correlations re-examined, with results given in appendices E11 & E12. A similar pattern of correlations were seen when the results were combined

and these will subsequently be discussed.

6.4 COMPARISON OF PARAMETERS FOR FIT v FRAIL GROUPS

6.4.1 All Subjects

Subjects were divided into fit or frail groups according to definitions given in appendix A1 and compared, with results given for the entire group in appendix E13, and Figs. 6.4.1 to 6.4.8.

Of the 49 subjects participating in the study, 25 were frail. Fit & frail groups were matched for age & DIG dose, but were significantly different in terms of mobility score. The fit passed a significantly greater urine volume than the frail group (1517±520 fit, 1216±780 fr.;$p<0.01$), and also weighed significantly more ($p<0.05$), with a greater SA ($p<0.05$).

DIG dose & [SDIG] were not significantly different between groups although the frail group exhibited a greater mean SDIG level and levels also tended to be higher when those taking similar dosages were compared (Figs. 6.4.1-6.4.3).

UDIG, [UDIG] and %Du were not significantly different between fit & frail groups (%Du: 46±6 fit, 47±19 fr).

Total clearance was significantly greater for the fit group (133±67 fit, 89±52 fr.;$p<0.02$) and this difference remained when Clt was expressed per kg bodyweight or normalized to $1.73m^2$ SA.

Renal clearance also tended to be greater in the fit group (86±43 fit, 65±48 fr.;$p<0.06$) but this trend became less distinct for Clr/kg ($p<0.2$) & Clr/SA ($p<0.2$), although minimum, maximum, Q1 & Q3, median & mean values remained consistently lower in the frail group.

Clnr was not significantly different between groups.

UCr was significantly greater in the fit group (1170±371 fit, 915±625 fr.;$p<0.02$) but [UCr] was not. SCr did not differ significantly between groups but frail subjects showed a greater range (0.55±1.66mg/dl fit, 0.34±2.69mg/dl fr). CCr & CCr/SA were both significantly greater for the fit group (CCr/SA: 83±24 fit, 63±37 fr.;$p<0.002$).

6.4.2 Female Subjects

26 female subjects participated in the study and 14 were frail. Results from this group are shown in appendix E14. Fit & frail groups of female subjects were matched for age (81±5y fit, 83±7y fr) but fit subjects were significantly more mobile ($p<0.001$).

Urine volume was significantly greater for the fit group (1488±423ml fit, 833±210ml fr.;$p<0.001$) while groups were similar for weight & SA ($p<0.2$).

DIG dose was similar between groups but the mean [SDIG] tended to be greater in the frail group (0.63±0.48 fit, 1.15±0.81 fr).

Neither UDIG or %Du were significantly different between groups but [UDIG] was statistically greater ($p<0.04$) for frail subjects due to the reduced volume of urine passed.

Clt was significantly greater for the fit group (112±43 fit 80±59 fr.;$p<0.04$), with the difference tending towards significance for Clt/SA ($p<0.06$), but being lost for Clt/kg. For both Clt, Clt/SA & Clt/kg, Q1, Q3, median & mean were greater for the fit than frail group.

No statistical difference was seen between Clr, Clr/kg & Clr/SA for the fit & frail female groups, but in all cases the fit tended to have greater values than the frail group, for minimum, maximum, Q1, Q3, median and mean.

Clnr was similar between groups.

UCr was significantly greater in the fit group (939±314 fit 712±569 fr.;$p<0.04$), but [UCr] was not. SCr exhibited a greater variability in the frail group, while both CCr & CCr/SA were significantly greater for the fit group (CCr: 73±16 ml/min fit, 54±40ml/min fr.;$p<0.02$).

6.4.3 Male Subjects

Of the 23 male subjects in the study, 11 were frail. Results from fit & frail male groups are shown in appendix E15. Groups were matched for age (77±7y fit, 79±6y fr) but fit subjects were significantly more mobile ($p<0.001$).
Fit & frail groups passed similar urine volumes although the fit group tended to weigh more and have a greater SA. There was no statistical difference between DIG dose or [SDIG] for fit & frail groups - %Du, UDIG & [UDIG] were also similar.
Clt, Clt/kg & Clt/SA were not statistically different between groups of subjects, but again fit subjects had greater values of minimum, maximum, Q1 & Q3, median & mean. There was no significant difference between Clr, Clr/kg & Clr/SA for the groups, but again Q1, Q3, median & mean tended to be greater in the fit group.
Median & mean Clnr & Clnr/kg were greater in the fit group but this was not significant.
UCr & [UCr] tended to be greater in the fit group. Although the range for SCr was wider in the frail group there was no significant difference between fit & frail groups. Both CCr & CCr/SA were statistically greater for the fit males (CCr: 96±34ml/min fit, 66±26ml/min fr.;$p<0.025$).

Fig. 6.4.1 Distribution of [SDIG] Levels in Fit & Frail Elderly People Taking 62.5mcg DIGOXIN Daily

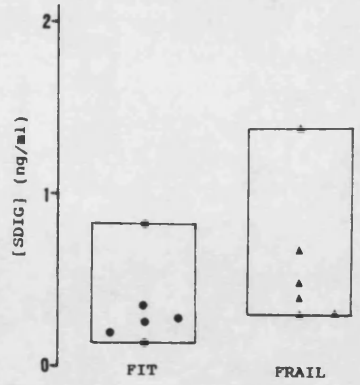


Fig. 6.4.2 Distribution of [SDIG] Levels in Fit & Frail Elderly People Taking 125mcg DIGOXIN Daily

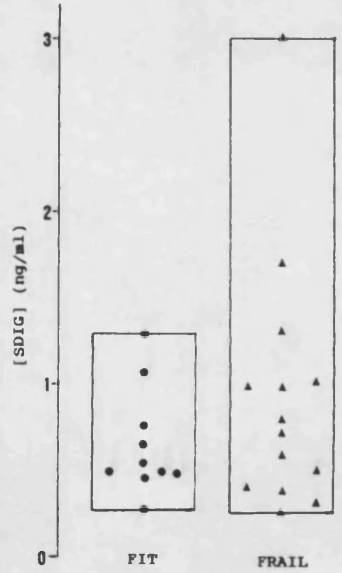
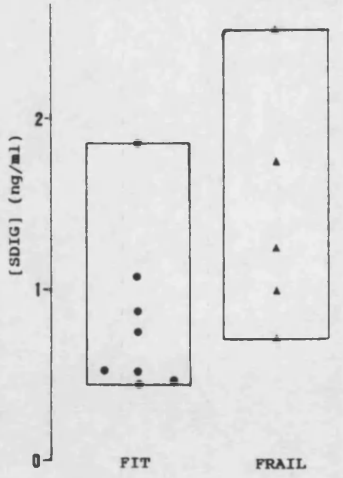


Fig. 6.4.3 Distribution of [SDIG] Levels in Fit & Frail Elderly People Taking 250mcg DIGOXIN Daily



● = fit subject ▲ = frail subject

Fig. 6.4.4 Distribution of %Du in Fit & Frail Elderly People Taking DIGOXIN

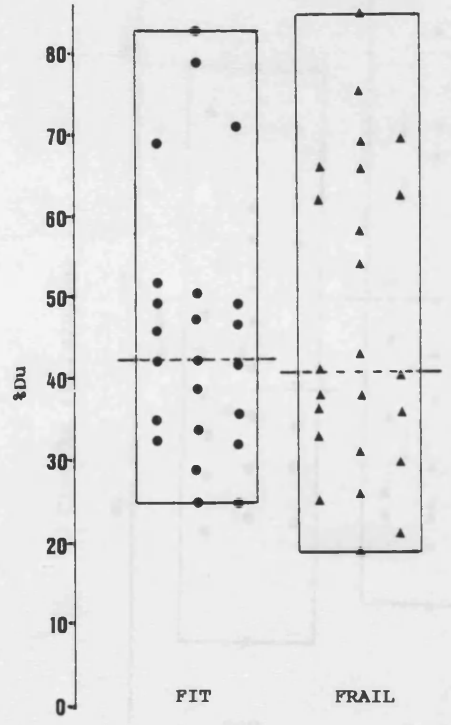
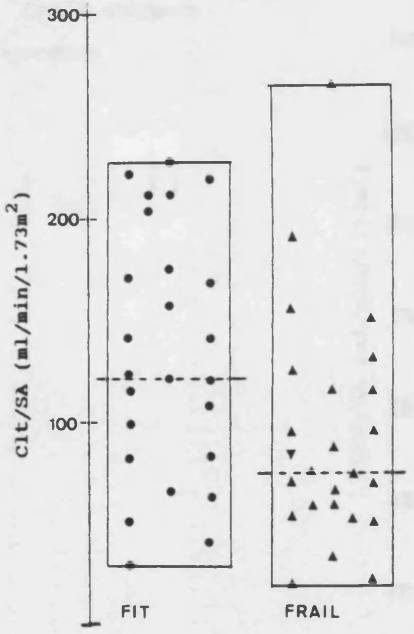


Fig. 6.4.5 Distribution of Clt/SA in Fit & Frail Elderly People Taking DIGOXIN



● = fit subject

▲ = frail subject

----- median

Fig. 6.4.6 Distribution of Clr/SA in Fit & Frail Elderly People Taking DIGOXIN

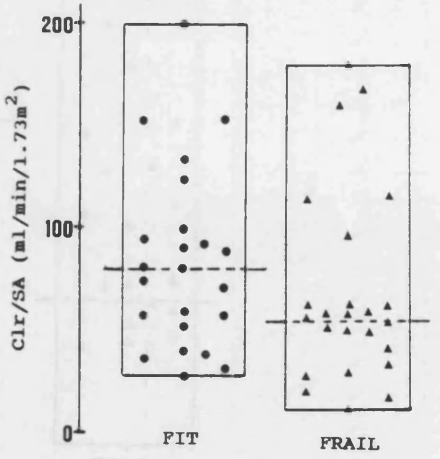


Fig. 6.4.7 Distribution of Clnr/kg in Fit & Frail Elderly People Taking DIGOXIN

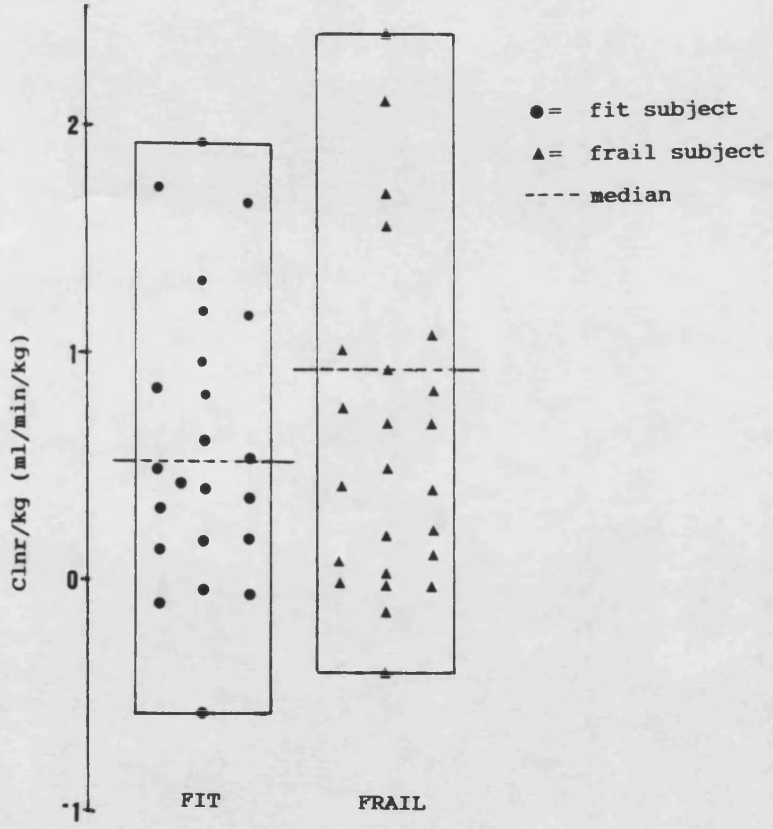
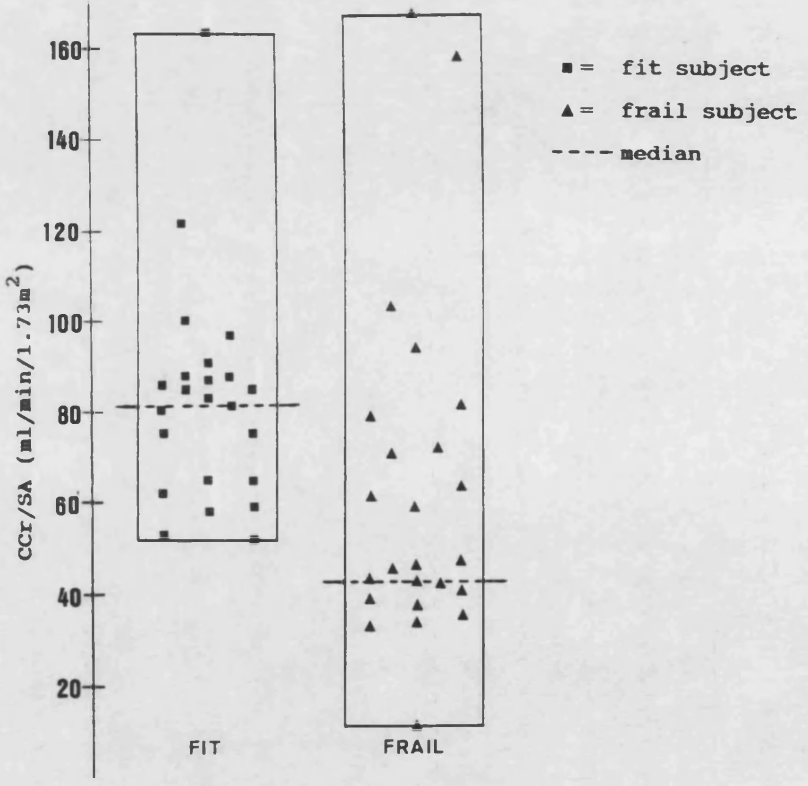


Fig. 6.4.8 Distribution of CCr/SA in Fit & Frail Elderly People Taking DIGOXIN



6.5 DISCUSSION

6.5.1 Digoxin Dose and Usage

DIG elimination was followed in a heterogeneous group of elderly people prescribed DIG chronically for a variety of cardiovascular conditions, and the influence of fitness and mobility on DIG excretion was investigated.

24 fit & 25 frail elderly people aged 66-96y completed the study. From the medical notes 33 subjects (67%) had been prescribed DIG for AF with or without CCF or LVF, and of those 30 were in AF during the study. Of the remainder, 5 (10%) were taking DIG for CCF or LVF alone, 5 (10%) for other cardiac conditions and 6 (12%) for no obvious reason. Clinicians agree that a fast ventricular rate in the presence of an irregular supraventricular tachyarrhythmia is a definite indication for DIG therapy. However, the role of DIG in the treatment of CCF alone remains controversial, and DIG is now rarely the treatment of choice for this condition (42,85,88,233). If both AF & CCF are justifiable indications for DIG therapy 22% of patients studied were probably prescribed DIG unnecessarily.

Reports on drug usage have frequently shown DIG therapy to be questionable, particularly in the elderly, but the incidence of inappropriate prescribing in this study compares favourably with others. One study in 1977 showed 24 patients registered with a General Practice to be taking DIG, 18 of whom were in sinus rhythm (84). Another study reviewed medical records of 150 outpatients prescribed DIG and found 42% taking DIG for a questionable reason (224). Prevalence of DIG prescribing in 5 Irish nursing homes was found to be 21%, with the prescription inappropriate in 48% of patients (225). Prevalence of DIG prescribing in English

nursing homes was found to be 11.5% (226). Another recent report from Canada found that 14% of nursing home residents took DIG, with the prescription seeming inappropriate in 70% of cases (227). Conversely, another study found DIG therapy to be questionable in only 4 out of 77 elderly patients (228). These subjects had recently been discharged from hospital after acute admission and this may explain the low incidence of unnecessary DIG.
Most of the above studies subsequently withdrew DIG where the drug seemed inappropriate, and few patients suffered a deterioration in health necessitating restitution of DIG therapy (84,98,225,227,228,229).

In this study subjects were taking DIG at a daily dose of 0.0625mg (n=12), 0.125mg (n=24) or 0.25mg (n=13; 0.78-5.81 ug/kg/day). No subject was taking alternate day therapy, or a combination of tablet strengths. Dosages are lower than those recommended by the manufacturer (0.25-0.5mg/day for normal CCr, 0.125-0.25mg daily in the elderly; 46) or British National Formulary (0.125mg daily in the elderly; 45). Others recommend a dosage of 2-5ug/kg/day depending on renal function (44) or 1.6ug/kg daily for those with end-stage renal failure (103).
DIG dosage has steadily declined in recent years as reports have suggested an increase in mortality & morbidity associated with its use (230). A dose of 0.0625mg daily has become a popular "geriatric dose" because of these fears and increasing awareness that DIG clearance is reduced in old age (91). While toxicity is unlikely from a daily dose of 0.0625mg, a large proportion of patients are under-digitalised as a result, and this dose is only appropriate in those with severe renal impairment or much reduced

bodyweight. The use of inadequate doses are of little therapeutic consequence in patients with sinus rhythm, when the prescription is often unnecessary. However, in renal failure & hypokalaemia patients may be exposed to toxicity. In patients with AF the apparent widespread use of inadequate doses may be cause for concern (225).

6.5.2 Serum Digoxin Levels

[SDIG] levels obtained for each DIG dose are shown below:-

DIG dose (mg)	n	[SDIG] (ng/ml)
0.0625	12	0.14 - 1.38
0.125	24	0.28 - 3.00
0.250	13	0.44 - 2.50

No statistical difference was seen between sexes.
The therapeutic range for DIG is 0.80-2.00ng/ml, although many clinicians aim to maintain [SDIG] between 1.0-1.5ng/ml (44,98). While the preferred range is unaltered in old age (90) & renal failure (103,231), control of supraventricular dysrhythmias may require a [SDIG] level of up to 3ng/ml, when the therapeutic benefit from control of heart rate offsets the increased probability of side effects.
From [SDIG] alone, subjects found to be subtherapeutic, therapeutic or toxic in this study are given below:-

DIG dose/mg	subtherapeutic	therapeutic	toxic
0.0625	10	2	0
0.125	15	8	1
0.250	6	6	1
Total	31(63%)	16(33%)	2(4%)

From clinical observations of heart rate and appearance of side effects, 5 subjects appeared to be subtherapeutic

(F01, M25,F33,F44,F52) and 1 toxic (F37) with AF. The prescription of DIG was also questionable in some cases, particularly in the absence of AF when [SDIG] was subtherapeutic. In such cases DIG can often be withdrawn (84,98,225,228,229). Signs & symptoms of toxicity are dose related (94), with [SDIG] above 3ng/ml, or a daily maintenance dose in excess of 0.7mg or 6ug/kg, invariably associated with side effects (89). However, studies have shown there to be considerable overlap in the [SDIG] at which toxicity occurs when [SDIG] may be as low as 1ng/ml (85,103,228).

Although [SDIG] is generally thought to be a useful diagnostic aid where over or under-digitalisation are suspected, some groups have proposed that knowledge of [SDIG] adds little to the clinical picture when the physician already has information on DIG dose, renal function, serum K^+ & cardiac status (232,233). Evaluation of the use and outcome of [SDIG] assays have also shown that few are carried out in appropriate circumstances (86). [SDIG] can only be interpreted in the clinical context since numerous other factors need to be taken into account including thyroid & renal function & electrolyte balance (85,86,89,233,234).

These results suggest that a dose of 0.0625mg daily is unlikely to produce [SDIG] levels within the therapeutic range except in severe renal impairment; those with AF and subtherapeutic levels (n=22) may have benefitted from an increased dose and those without AF whose [SDIG] was below 0.8ng/ml (n=9) could probably have been withdrawn from DIG. Other studies have also drawn similar conclusions where a substantial proportion of underdigitalisation has been

found following a daily dose of 0.0625mg (225,235,236). A daily dose of 0.125mg is more appropriate in the elderly and carries only a small risk of toxicity but 0.1mg tablets would be a useful alternative. The recommendation of 0.25mg daily in the elderly with CCr greater than 25ml/min (102,237) may be too high for those with a low bodyweight.

Toxicity is more likely to occur at a lower [SDIG] in patients with hypothyroidism, hypokalaemia, hyperkalaemia or other electrolyte disturbances, where the myocardium is sensitized to DIG (89,92,98). Hypothyroidism renders patients more sensitive to DIG and side effects may occur within the therapeutic range (238,239). Conversely, hyperthyroidism is associated with DIG resistance where larger than usual doses are required to control supra-ventricular dysrhythmias and an increased GFR associated with this condition enhances renal clearance (237). The maintenance dose of DIG should always be reviewed when the thyroid dysfunction is brought under control.

In this study subjects with thyroid disorders were receiving treatment to render them euthyroid, but possible over or under treatment of the thyroid disorder could affect their DIG sensitivity. 4 subjects were hypothyroid (F04,F17,F21,F46), 2 were previously hyperthyroid (F33,M23) No hypothyroid subjects exhibited any signs of toxicity, but F33 appeared to be clinically subtherapeutic when taking 0.125mg DIG daily, giving [SDIG] of 1.03ng/ml but a pulse rate of 102bpm.

Both low (<3.5mmol/l) and elevated (>5mmol/l) serum potassium levels are associated with the appearance of DIG toxicity when [SDIG] is within the therapeutic range (89). Hypokalaemia can arise from diuretic therapy which is often

used in combination with DIG for the treatment of CCF. Other causes of low serum K^+ include poor nutrition, diarrhoea & vomiting and chronic wasting disease. In cases of suspected toxicity both serum K^+ & [SDIG] should be measured. No subject in the community had had a recent K^+ measurement despite 34 regularly taking a diuretic, whereas 1 patient in hospital (F17) had had K^+ measured & 2 patients had [SDIG] levels taken during their admission. Despite the recommendation that [SDIG] can only be interpreted in the clinical context, this appears to be rarely the case.

For a given dose [SDIG] was found to be highly variable, exhibiting an eleven-fold range for those taking 0.125mg daily. [SDIG] is influenced by dose, and for an individual an increase in dose is accompanied by a proportional increase in [SDIG], since DIG pharmacokinetics are independent of dose after single doses & at steady state (92,240,241). However, for any DIG dose a considerable interindividual variation in [SDIG] is seen, even within a group of patients with comparable CCr (242).
In this study [SDIG] correlated with dose, Clt & Clr for females; Clt & Clr for males; dose, UDIG, Clt & Clr for the entire group. In all cases [SDIG] & CCr appeared to be correlated with a significance level of $p<0.05$. These correlations are in agreement with those reported by other groups (236,240). Some have subsequently attempted to devise a nomogram or equation to enable [SDIG] to be predicted for given dose & renal function (103,243,244,245) while others have constructed a scoring system to aid selection of an appropriate DIG dose (246). Investigations have shown that while dose & CCr are the most important

determinants of [SDIG], plots of measured v predicted [SDIG] consistently yield a degree of unexplained variance, suggesting that other factors need to be taken into account (89,91,92,103,236,240,242). Incorporation of additional parameters such as age, sex, lean body mass & [albumin] into equations has not been shown to significantly increase the accuracy of prediction with the degree of scatter remaining clinically unacceptable (105,113,184,235,245,247, 248); in one instance calculated [SDIG] level overestimated actual measured level in 33% of patients studied (242). Substantial interindividual variation in [SDIG] may be due to differences in one or more of the following aspects of DIG disposition: absorption, distribution, protein binding, drug interactions, diagnoses, metabolism, elimination or compliance. The way in which these factors can alter DIG disposition in old age & ill health are discussed in 1.4.3.

6.5.3 Total Body Clearance of Digoxin

Total body clearance was calculated from [SDIG] & fraction of dose absorbed (67%) and found to range from 19-224ml/min (95±54) for females, 34-264ml/min (128±69) for males. Many other studies have calculated DIG elimination half-life or [SDIG], but where Clt has been determined results are similar to those in the present study. One study (91) found mean Clt to be 37±6ml/min for elderly subjects (age 81±2y) and 106±14ml/min for younger subjects (47±5y). Another calculated Clt to be 109±17ml/min for a group of elderly males (78±2y), assuming 95% absorption (242).

No statistical difference was seen for Clt, Clt/kg & Clt/SA between females & males, suggesting that DIG excretion is similar in both sexes. This has also been concluded in other studies (184,247), although the prediction of [SDIG]

has been found to be more accurate for females (228,244).

In order to calculate Clt it was assumed that all subjects absorbed an equal fraction of the DIG dose administered, but this cannot be proven from data in this study. For a drug in steady state, the extent, but not the rate, of absorption is important in determining the concentration of drug in the blood at all times (44). While age per se has not been shown to affect the extent of drug absorption (including DIG) via simple diffusion, certain disease states have (9,12,13,91,235). Therefore, the data is interpreted with caution.

Clt correlated with mobility score, SCr, CCr, [SDIG], Clr & Clnr for females; CCr, dose, [SDIG], Clr & Clnr for males; age, mobility score, SCr, CCr, [SDIG], Clr & Clnr for the entire group. Previous studies have shown DIG Clt to be reduced in old age (91), as seen in the present study for the entire group despite the relatively narrow age range examined (66-96y). Age is associated with a decline in CCr and this was seen in the present study. While a definitive statement cannot be made on the relative importance of age & CCr in Clt determination, it would seem that CCr is the more important when correlation coefficients are observed. Females: age v CCr r=-0.375; age v Clt r=-0.426; CCr v Clt r=0.659. Males: age v CCr r=-0.504; age v Clt r=-0.453; CCr v Clt r=0.573.

An increase in mobility score was significantly correlated with a decrease in Clt for the females & entire group, tending towards significance for the males. In this study mobility score was evenly distributed throughout the age range so that no correlation was seen between these two

parameters. However, a correlation was seen between mobility score & CCr, known to strongly influence Clt, and it is again difficult to separate out the importance of these two factors.

The effect of chronic exercise on DIG kinetics has not been investigated since it would involve chronic dosing to healthy subjects. However, the effect of acute exercise on DIG distribution has been examined in two studies. One study measured [DIG] in thigh muscle & serum following two weeks of dosing with DIG, before & after one hours exercise (249). Increased binding of DIG to muscle was seen immediately following exercise accompanied by a reduction in [SDIG]; 30 minutes later [SDIG] had increased, but not to pre-exercise levels. The ratio [muscle DIG]/[serum DIG] was greater after exercise suggesting that mobilization of DIG from other tissue compartments was responsible for the increased [DIG] in muscle observed after exercise. The second study however, failed to show a change in [SDIG] following a period of maximal exercise (250).

Changes in posture & physical activity have marked effects on cardiac output & plasma volume and cause alterations in the concentration of nonfilterable constituents, although the extent to which these changes influence drug disposition has been given little attention (171,251). One study compared [SDIG] in 8 healthy volunteers after 2h of normal physical exercise with that after 2h of recumbency (171). Rest in the supine position was associated with a relative increase in [SDIG] of 63% although interindividual variation was considerable (28-91%) while urinary excretion of DIG remained unchanged. The combined effect of these observations was a decline in Clr although CCr remained

unchanged. Recumbency has been shown to produce 7-8% increase in plasma volume which cannot account for the increase in [SDIG]. A reduced Clr could be the result of a lowered tubular secretion associated with a decline in renal blood flow at rest. Alternatively, changes in DIG binding to muscle could be responsible for the increased [SDIG] since muscle binds about 60% of the body store of DIG, any change in binding can have a profound effect on [SDIG].

A second study investigated the effect of rest in the supine position on [SDIG] in outpatients taking DIG chronically (252). Rest was associated with a rise in [SDIG] which appeared to reach "steady state" after 2h. [SDIG] increased by a mean of 28% although again the inter-individual variation was great (0-75%). This suggests that everyday physical activity may affect [SDIG], but in an unpredictable and variable fashion.

In the present study all subjects took DIG in the morning, and [SDIG] was measured 11h post-dose after a full day of activity or inactivity. It is possible that the level of activity, as rated using the mobility score, and posture, may have influenced [SDIG]. If DIG binding to skeletal muscle is greatest in those who were most active (lowest mobility score) this would have the effect of reducing [SDIG] & increasing Clt, as seen in the present study. Stimulation of renal blood flow by physical activity could also enhance Clr.

It is possible that mobility alone can influence Clt, although to what extent cannot be determined. When subjects were ranked in order of decreasing CCr a trend was seen towards a reduction in Clt. However, one subject with a

CCr of 34ml/min/1.73m^2 had a Clt of 1.97ml/min/kg, while another with a CCr of 97ml/min/1.73m^2 had a Clt of 1.28ml/min/kg. It is apparent that while there is a statistically strong correlation between CCr & Clt, other factors besides CCr are important.

Other studies which have investigated the effect of CCr on Clt using patients with renal failure have also found a degree of unexplained variance (105). Mobility may be an additional factor in the determination of DIG Clt, but it seems likely that any influence is small.

The variation in Clt may alternatively be explained by an unequal extent of absorption occurring between subjects. The effects of disease states & drug interactions on DIG absorption have been discussed in 1.4.3. At steady state the amount of drug absorbed is equal to that excreted.

In this study the percentage of dose excreted unchanged in urine was variable, ranging from 19-83% (45 ± 19) for females & 25-85% (48 ± 16) for males; 8 subjects had more than 67% unchanged DIG in urine. Possible reasons for this could include increased absorption, differences in compliance or lack of specificity of the assay. Extent of DIG absorption has been reported to vary between subjects but most studies were carried out before the standardization of DIG tablets, when variation in bioavailability was partly due to differences in dissolution rate. Comparative studies using i.v. and oral formulations would be needed to determine whether absorption in elderly people was constant. It remains possible that the extent of absorption may have varied among the population studied, although it is not possible to say by how much. A low %Du may be indicative of more extensive metabolism and non-renal elimination.

6.5.4 Renal Clearance of Digoxin

Renal clearance was calculated from [SDIG] & DIG excreted unchanged in urine in 24h and varied from 10-171ml/min (66±47) for females, 27-194 ml/min (85±45) for males. The ratio of renal clearance of DIG/renal clearance of creatinine, Clr/CCr, varied from 0.33-2.46 (1.09±0.56) for females, 0.55-1.79 (1.03±0.34) for males. In another study, mean Clr was found to range from 83-119ml/min/1.73m^2 in healthy volunteers & 53-73ml/min/1.73m^2 in elderly patients with heart failure; chronic renal failure was also seen to be associated with a reduced Clr (92). A second study found Clr to vary from 4-57ml/min, with a mean Clr/CCr of 1.04, in a group of elderly people aged between 65-94y (237).

In many subjects renal excretion of unchanged DIG is the most important route of elimination. Nomograms & equations for [SDIG] prediction usually include information on CCr since most of that DIG renally excreted is via glomerular filtration of which CCr is an estimate (chapter 2). Using data from the present study, a plot of CCr v [SDIG] for each DIG dose gave a straight line for the majority of subjects, and a high CCr was associated with a low [SDIG]. However, a small but significant proportion of subjects fell outside this relationship, usually with a greater than expected [SDIG] given their CCr. This considerable scatter has been noted in other studies (253), causing difficulties with prediction of [SDIG] as previously discussed. When DIG half-life is plotted against CCr a similar variation has been seen (92,93).

These results suggest that clearance of DIG & creatinine are related and may possibly be excreted via a common pathway. However, the wide range in Clr/CCr seen in this

study suggests that this relationship does not hold for all subjects. Clr was found to correlate with SCr, CCr, %Du, [SDIG] & Clt for females; CCr, UDIG & Clt for males; age, mobility score, SCr, CCr, %Du, UDIG, SDIG & Clt for the entire group.

Previous studies have also shown Clr to be reduced in old age (91,237) probably due to the inevitable decline in CCr seen with advancing years, since CCr & Clr are closely related in most cases. Again it is not possible to say whether mobility asserts an additional effect on Clr or whether the correlation is the result of the relationship between CCr & mobility. The effect of exercise on the renal excretion of DIG has not been fully investigated, but it seems likely that CCr is more important than mobility, which may assert a small additional influence on Clr.

6.5.5 Non-renal Excretion of Digoxin

When renal clearance is subtracted from total body clearance the product represents that DIG which is cleared by other "non-renal" routes. This DIG may either be excreted unchanged by an alternative route eg. secretion into bile, or else metabolized to form a compound that can be renally eliminated or excreted by another route. It is now thought that nonrenal routes of excretion are responsible for the elimination of approximately 20-40% of the administered dose although metabolism appears to be more extensive in about 10% of those taking DIG (254,255).

In this study Clnr ranged from -30-105ml/min for females & -35-130ml/min for males; 8 subjects had a negative value for Clnr (Clr>Clt). This suggests that some subjects may have absorbed in excess of 67% of the dose. An increase in

DIG available for systemic circulation would have the effect of increasing Clt to create a positive value for Clnr. Lack of specificity in the DIG assay could also account for this result if other compounds in serum or urine cross-reacted with the DIG antibody.

Clnr for most subjects was calculated to be greater than zero, suggesting that not all DIG was eliminated unchanged in urine. The range for Clnr suggests that the importance of the non-renal route(s) may vary between individuals, and it has been proposed that DIG metabolism may increase during chronic therapy (111). Most subjects studied had been taking DIG for a number of years - only one subject (M55) had recently been prescribed DIG and 3 weeks had elapsed before inclusion into the study. Compromised renal function is often associated with an enhancement of non-renal routes of elimination. However, in this study Clnr was independent of both CCr & Clr, suggesting that renal function does not determine Clnr. Similar conclusions have been drawn by other groups investigating DIG metabolism in those with renal impairment (110), and faecal excretion of DIG and metabolites has not been found to alter greatly when renal function is diminished (256). It has also been shown that the total body clearance of DIG, which includes both renal & extrarenal clearances, is in a linear relationship with renal clearance of creatinine (104). The pattern of correlations suggests that mobility is not a factor in the determination of Clnr, and cannot explain the variation in Clnr observed in this or other studies.

6.5.6 Urinary Excretion of Digoxin

Since subjects in this study were taking a variety of doses, DIG excreted in urine in 24h was expressed as a

percentage of dose administered. %Du ranged from 19-83% for females, 25-85% for males, and there was no significant difference between sexes. Other studies have found %Du to vary from 12-69% (42±4) in a group of 20 elderly people (237), and to average 36±28% in 53 hospitalized elderly people with a mean age of 72y (236). The latter study noted that neither a variation in weight nor co-administration of diuretics affected %Du, but a decrease in CCr was associated with a reduction in %Du. Another study found mean %Du to be 59±6% (range 38-82%) in a group of elderly people with heart failure taking good bioavailability tablets (257).

In the present study %Du correlated with Clr & Clnr for females; Clnr for males; UDIG, Clr & Clnr for the entire group. Neither age, mobility score or CCr appeared to influence %Du, but Clr appears to be an important determinant. Those with a low %Du could either have absorbed less DIG or metabolized a relatively greater proportion of the dose. It has been estimated that around 10% of those taking DIG are subject to reduced absorption due to the action of various enteric organisms (85). An additional 10% exhibit a more extensive metabolic pathway when between 20-55% of the dose is excreted as a DIG metabolite (98). The degree of metabolism is probably not related to renal function, which is in agreement with the findings from this study where no correlation was seen between %Du or Clnr & CCr.

Whenever a 24h urine collection is required, there are inherent problems in obtaining an accurate collection, except when patients are catheterised. Males generally have less problem collecting urine, but no difference was

seen between sexes for %Du, suggesting that collections were comparable in accuracy between males & females. Inaccuracy of urine collection cannot explain the results from those individuals having a greater than average recovery.

6.6 DIGOXIN EXCRETION IN FIT & FRAIL ELDERLY PEOPLE

6.6.1 Sex Difference

Subjects were divided into fit & frail groups which were further separated into males & females. Males were less elderly than females but this was not statistically significant. Trends between fit & frail groups were similar when sexes were separated, and %Du, [SDIG], Clt, Clr, Clnr were not significantly different for males & females. Fit & frail groups are therefore of mixed sex except were specific differences were seen (UCr, CCr, weight, SA). These will be discussed under each heading where applicable.

6.6.2 Comparison of Age & Mobility Score

Data were collected from 24 (12F) fit & 25 (14F) frail subjects taking DIG long-term. Mobility score was evenly distributed throughout the age range, so that while no significant difference was seen between ages of fit & frail groups, mobility score was significantly greater in the frail group. There was no overlap between groups with respect to mobility score - fit subjects scored 1 (n=17) or 2 (7), frail subjects scored 3 (13), 4 (8) or 5 (4). To achieve an even distribution of scores throughout the age range it was necessary to extend recruitment of subjects beyond the one General Practice and hospital used in previous studies. Patients were therefore recruited from

an additional 5 General Practices & 4 Community Hospitals. Although the time taken for recruitment was increased, it enabled fit & frail groups to be comparable for age so that the effects of other factors such as frailty & CCr could be more clearly elucidated.

6.6.3 Comparison of Digoxin Dose & [SDIG]

Subjects were prescribed 0.0625, 0.125 or 0.25mg DIG daily, with no significant difference seen between fit & frail groups in terms of daily dosage or dose per kg bodyweight. [SDIG] tended to be greater in the frail group, who exhibited a wider variation for a given dose, but these observations failed to reach significance. As discussed in 6.5.2 the determination of [SDIG] is complex, and frail could differ from fit subjects at any stage of DIG disposition.

Reduced absorption of DIG in fit subjects could explain the lower [SDIG], particularly if they consumed a diet higher in bran fibre or fat both of which have been shown to impair absorption of DIG. However, %Du was similar between groups suggesting that both absorption & metabolism are comparable.

Vd of DIG has been shown to be reduced in the elderly (106) but similar within a group of elderly subjects (237), although the effect of frailty is not known. No significant difference in weight or SA was seen between fit & frail groups when separated according to sex while the trend seen for [SDIG] remained. Body composition can influence DIG distribution, and fit subjects would be more likely to have a greater proportion of muscle as indicated by an increased UCr, which could reduce [SDIG]. Frailty is likely to be associated with a greater degree of muscle wasting.

Polypharmacy is most likely to occur in frail subjects who generally have multiple pathology requiring numerous drugs. 19 frail & 2 fit subjects were in care during the study. It is possible that those at home took DIG less regularly than those in care who are more likely to have their medications supervised. This could account for the reduced [SDIG] seen in fit subjects, and while the blood samples taken 5 days apart appeared to indicate that all participants were in steady state, this cannot be proved unequivocally. Polypharmacy among the least fit could give rise to more drug interactions which could increase [SDIG] in the frail elderly.

The most significant differences between the fit & frail groups were those of mobility score & CCr. In 6.5.3 the relationship between CCr & mobility was discussed and it was difficult to say whether mobility independently contributed to the [SDIG] & clearance. Males had a significantly greater CCr than females although the difference was reduced for CCr/SA. For both sexes CCr & CCr/SA were significantly greater in the fit than frail groups. CCr appears to be the most important factor in the determination of [SDIG] and it seems likely that this would explain at least some of the difference observed in [SDIG] between fit & frail groups.

6.6.4 Comparison of Excretion of Digoxin In Urine

Urine volume & UCr excreted in 24h was significantly greater in males than females, and so fit & frail groups compared were of separate sexes. Urine volume & UCr were significantly greater in the fit group of females. A similar trend was seen for UCr in males, but urine volume was comparable between groups. While it is possible that

urine collections were incomplete for frail groups, UCr would be expected to be greatest in those who are most active, since the quantity of creatinine excreted is related to muscle mass.

Range & mean %Du were similar between fit & frail groups, for both sexes, suggesting that frailty, immobility or reduced CCr did not significantly influence the quantity of unchanged DIG excreted in urine. It would seem that the extent of both DIG absorption & metabolism did not differ significantly between fit & frail groups, otherwise % recovery of unchanged DIG in urine would be expected to be different between groups.

6.6.5 Comparison of Digoxin Clearance

Total body clearance could not be calculated since DIG was administered orally and the exact fraction of dose absorbed was not known, and so apparent body clearance was instead determined. Renal clearance was calculated from [SDIG] at steady state & DIG in urine, and therefore did not require such assumptions to be made. Clnr was taken to be the difference between Clt & Clr.

The fit had a significantly greater Clt than the frail group when the entire group and females were considered, and this observation tended towards significance for males. The significance of this relationship was reduced when Clt was expressed per kg bodyweight or $1.73m^2$ SA, but the trend could still clearly be seen.

Fit tended to have a greater Clr than frail for the entire group, males & females, but in all cases this trend failed to reach significance. Median & mean Clr, Clr/kg & Clr/SA were consistently lower in the frail groups, likewise Q1 & Q3. Clnr, Clnr/kg & the ratio Clr/CCr were similar for

each group. The observed difference in Clt between fit & frail groups could be due to either an unequal extent of absorption, difference in CCr, or influence of frailty. Since clearance is independent of Vd, changes in body composition cannot explain the differences seen. In 6.6.4 %Du was discussed in relation to fit & frail groups. While urine volumes differed between groups %Du did not, suggesting that absorption & metabolism were similar between groups, even though extent may vary between individuals. Both Clt & Clr correlated with CCr, as in other studies, since in most individuals the majority of DIG is eliminated by glomerular filtration and to a lesser degree tubular secretion.

CCr was significantly different between fit & frail groups and this is likely to explain some of the observed differences in Clt & Clr. Although those with the lowest CCr tended to have the lowest Clt & Clr values, this was not unequivocally so, and this degree of variability also seen in other studies confounds any predictive method for [SDIG] based on CCr. The ratio Clr/CCr was similar between groups, and close to unity, suggesting that creatinine & DIG are cleared by a common pathway. Clnr was comparable between groups suggesting that the degree of non-renal elimination is unchanged in the frail state. Other studies have found that Clnr is similar for a range of renal functions and results from the present study are in agreement with these findings (104,256).

From these results it would appear that the association between frailty & CCr is the most likely cause of the trend towards a reduced Clt & Clr seen in the frail groups. Since mobility score & CCr were correlated, it is difficult

to say whether frailty & immobility has an additional influence on Clt & Clr above that from CCr.

In an attempt to separate out the influence of frailty from CCr, sub-groups of fit & frail males were formed. Subjects were selected whose CCr/SA was within the range 45-105 $ml/min/1.73m^2$ and using this method subgroups of 11 fit & 8 frail males were formed. Results are given in appendix E16. The distribution of CCr & mobility score amongst females did not facilitate the formation of fit & frail subgroups for females, and sexes were not combined since males had a greater CCr than females.
No significant difference was seen between fit & frail males with respect to age, dose, CCr & CCr/SA. With the equalization of CCr between fit & frail groups no significant difference was seen between groups for [SDIG], Clt, Clr or Clnr. These results would suggest that frailty & immobility exert little, if any, additional influence on the determination of DIG clearance. CCr appears to be the most important of those factors measured, and appears to be reduced in frailty. The unexplained variance observed in the prediction of [SDIG] is unlikely to be accounted for by the fitness of the subject.

6.7 CONCLUSIONS

The elderly are frequently prescribed digoxin because of an increased prevalence of the two primary indications for digoxin therapy, namely atrial fibrillation and congestive cardiac failure, associated with old age (242,258). However, widespread use of the drug has resulted in rates of toxicity which can approach 20% of hospitalized geriatric patients taking digoxin (259). There is also a

significant incidence of underdigitalisation (91,225), and this is becoming more common as digoxin doses fall (230) following fears of increased mortality and morbidity associated with its use.

DIG has a narrow therapeutic ratio and many studies have been carried out in an attempt to accurately predict serum [DIG]. Many factors have been found to influence [SDIG] but the scatter between measured and predicted [SDIG] remains wide, and the unexplained variance has rendered the use of nomograms and equations clinically unacceptable. While dose & renal function appear to be the most important determinants of [SDIG], other factors which also contribute include extent of absorption, distribution, metabolism & excretion, also disease state, compliance and drug interactions as discussed in 6.5.

The aim of the present study was to determine whether immobility & frailty exert an additional influence on the determination of [SDIG] and efficiency of elimination.

When patterns of correlations were examined many of the parameters studied were interrelated, and it was hard to make a definitive statement on the importance of mobility. Results appeared to indicate that if mobility does influence the efficiency of DIG elimination, then that influence is only slight and of minor significance compared to that of renal function. This suggests that incorporation of a "mobility factor" into an equation used to predict [SDIG] would not significantly enhance prediction accuracy and account for a degree of unexplained variance.

When subjects were divided into fit & frail groups a similar conclusion was drawn. The fit group had a greater total body clearance & renal clearance than the frail group

but this was confounded by the increased CCr seen in fit subjects. Males were divided further into fit & frail groups, each with a similar CCr range, and clearances re-examined. It was found that the trend towards an increased clearance in fit subjects was lost, with Clt & Clr becoming comparable for both groups. This suggested that the differences observed between fit & frail groups could partially be explained by the difference in CCr.
Thus while frailty, as defined for the purposes of this study, cannot be proposed as an explanation for the variability in [SDIG], the difference in renal function between fit & frail subjects should be considered. Many of the equations devised to predict [SDIG] also predict CCr from SCr and do not take into account frailty. This may explain some of the inaccuracies seen when nomograms or equations are employed. Nevertheless, when CCr is measured and plotted against [SDIG] or Clt a straight line is not seen, suggesting that the difference in CCr is not solely responsible for the scatter. Further work is required to determine which other factors besides those already mentioned are important in the determination of [SDIG].

From this study it was observed that a significant proportion of subjects on DIG were underdigitalised, with 63% having [SDIG] less than 0.8ng/ml. It has previously been proposed that the decline in DIG dosage in recent years has been the result of numerous articles published which have found a significant degree of DIG toxicity in those taking DIG (225,230). The popular "geriatric dose" of 0.0625mg daily is inappropriate in all but the most severe cases of renal insufficiency or muscle wasting, and subtherapeutic [SDIG] levels can give cause for concern in

those with uncontrolled AF. However, toxicity is unlikely with this dose and would only be seen in patients exhibiting an enhanced sensitivity to DIG. A maintenance dose of 0.125mg daily would appear to be the most appropriate dose for elderly subjects, alternatively a 0.1mg tablet may increase the proportion of subjects with [SDIG] within the therapeutic range.
Further to this is the question of when the prescription for DIG is appropriate which remains a controversial issue. While it is agreed that DIG therapy is necessary in supraventricular tachyarrhythmias, its role in CCF remains uncertain. Results from the present study suggest that prescribing trends are changing so that fewer patients are prescribed DIG in the absence of AF. Nevertheless, a proportion of patients remain who have been prescribed DIG, often in small doses, for a number of years on the basis of an uncertain diagnoses. Review of patients in General Practice on long term DIG would seem to be appropriate, since all are at risk of toxicity, particularly if concomitant diuretic therapy results in hypokalaemia.

Despite the vast literature concerning patterns of DIG elimination, more work needs to be carried out to determine causes of extensive interindividual variability observed for both [SDIG] & clearances. Those patients on long term DIG should be regularly reviewed since therapy is not without hazard, and a change in clinical condition can force [SDIG] beyond the therapeutic range. If the prescription remains appropriate, then steps should be taken to ensure that the patient receives maximal therapy to adequately control the condition but minimize the possibility of toxic side effects.

CHAPTER SEVEN

CONCLUSIONS FROM STUDIES IN FIT & FRAIL ELDERLY PEOPLE

Demographic trends indicate that the proportion of elderly people in the community is likely to expand in developed countries in the foreseeable future. Prevalence & incidence of ill health and disability increases in old age and it is not therefore surprising that those over retirement age are the main consumers of prescribed medication. In this age group adverse drug reactions are known to be two to three times more common due to the increased drug consumption and altered drug response. Knowledge of these facts has stimulated research into age-related changes in drug handling in an attempt to reduce the incidence of adverse drug reactions and make drug usage in the elderly safer.

Far from forming a single homogeneous group, the elderly can readily be seen to differ widely in their physical abilities even in the absence of discernable disease. Some elderly people remain independent, exhibiting little loss of function, until well into their ninth decade, while others appear to require full care soon after retirement. Many studies have been carried out to identify and quantify physiological changes associated with increasing chronological age, and these have subsequently been extended to investigate age-related changes in drug disposition. However, most such studies have been carried out using healthy elderly volunteers who themselves are frequently ill defined, and it is not yet widely known whether ill health & immobility contribute an additional decrement to the decline in many physiological functions associated with ageing.

As discussed in 1.2, the first precise definition of the "healthy elderly" was made in 1976 in the "Senieur Protocol", the result of a concerted working party to establish admission criteria for immunogerontological studies (29). However, this definition was not subsequently employed in drug studies, probably because it is too exacting for widespread use, with multiple clinical, biochemical & haematological investigations required before recruitment into a study. The number of potential volunteers disqualified from participation in a study by the strict exclusion criteria set down in the Senieur Protocol would appear to be too great for practicable usage in the majority of studies.

An alternative operational definition was proposed by a second group in 1988 in an attempt to dissociate biological & chronological age, and the terms "fit" & "frail" were coined for the purpose (28); the exact definitions are given in 1.2. Whilst the definitions used by this group are much less strict than those in the Senieur Protocol, they nevertheless require both clinical examination & laboratory investigations. Frail subjects were dissociated from their fit counterparts by their loss of independence, impaired ability to carry out activities of daily living, reduced mobility and probable requirement for prescribed medication. Whilst frail subjects could suffer from certain musculoskeletal and cardiovascular conditions, both fit & frail subjects were required to be free from significant hepatic, renal, cardiac, respiratory or metabolic disorders to fulfil the admission criteria.

Using these later definitions studies have found the frail

elderly to have reduced mental function, reduced total body potassium & albumin, and alterations in other electrolytes when compared to their fit counterparts. While the fit elderly exhibit the normal biological reduction in hepatic volume & blood flow, the frail elderly appear to possess an additional decrement in hepatic drug clearance, possibly due to a reduction in the specific activity of some hepatic enzymes. However, it is not yet known whether the normal reduction in renal function observed in the fit elderly is exaggerated in frail patients to further reduce renal drug clearance beyond that expected.

The main shortfall in the use of these later definitions is that the majority of elderly subjects in both the community and in care are not included, since most have some significant pathology requiring regular medication. Thus, those elderly people with well controlled chronic conditions which do not adversely influence quality of life are excluded from possible investigation despite their appearance of "fitness". Important changes in drug disposition could potentially be missed in those groups of elderly people most commonly prescribed medication either in General Practice or hospital.

The primary aim of this thesis was to devise working definitions which could readily differentiate between the two groups of elderly people most commonly encountered in clinical practice, that is, those who are well & active despite the presence of a chronic condition, and those who are infirm & relatively immobile in the presence of similar diagnoses. The groups thus defined were termed "fit" & "frail" since these titles most appropriately describe the

two populations, and precise definitions are given in appendix A1.
Together the fit & frail groups appear to form the majority of those elderly people for whom medication is prescribed. The definitions were constructed in such a way as to be easily applied to an individual without reliance on laboratory investigations, and thus they are composed of social & functional criteria. In the course of the following studies few individuals were not readily categorized.

The main criticism of these operational definitions is that without the use of laboratory investigations undiagnosed pathology may lead to a frail subject being categorized as fit, although the converse is unlikely to occur. While this problem was recognised, the reduction in accuracy was felt to be more than offset by the ease of application of the definitions.

Once the main populations of elderly people were defined, studies were carried out to determine whether fit & frail groups of elderly people differed in terms of renal function, and renal & hepatic drug clearance using model drugs frusemide, digoxin & paracetamol.

It is often desirable to know a patients renal function when drugs are prescribed whose elimination is accomplished via renal clearance, in order to prevent their accumulation. Since renal function is known to decline in old age, the margin for safety is reduced for renally excreted drugs with a narrow therapeutic window.
Creatinine clearance, taken as an estimate of glomerular filtration rate, was measured in a wide range of elderly

people in order to determine whether drug dosage levels should take into account the "fitness" of an individual. Since the classical measurement of CCr is a difficult & lengthy procedure, equations to predict CCr have previously been constructed using SCr alone. Therefore, in addition to the usual 24h CCr measurement, CCr was predicted using 12 different equations, and also urine collections of less than 24 hours. Measured & predicted CCr were compared between groups of fit & frail elderly people to determine whether frailty compromised the accuracy of CCr prediction.

In addition to the fit & frail subjects invited to participate in this study from General Practice & long-stay hospital wards, elderly people were recruited from the R.I.C.E. volunteer panel. All were free from discernable disease on clinical examination & laboratory investigations, fully mobile, and living independently in the community, and none were taking regular medication. These subjects were termed "very fit" and this group was taken to be comparable to the healthy elderly groups defined by previous investigators.
When the CCr of the very fit group was compared with the CCr of those defined as "fit" for the purposes of the ensuing drug studies, no significant difference was found between them. This suggests that the fit group as defined in this thesis is similar to those fit groups defined in other studies, at least in terms of renal function and probably renal drug clearance. The advantage of the present fit definition is that subjects included in the admission criteria are more frequently found in the clinical setting and thus more truly representative of those elderly people active in the community.

A statistical difference was seen between the sexes in terms of UCr, SCr & CCr and so results from males & females were analysed separately although similar patterns were seen for both groups. For both sexes CCr was found to decline with increasing age. CCr was compared between the very fit, fit & frail elderly males & females, and found to be comparable between the very fit & fit groups. However, frailty appeared to contribute an additional decrement to an individuals CCr since frail groups had a consistently reduced CCr when compared to their fit counterparts of similar age & weight.

Using urine collections of about 8 hours, predicted CCr closely approximated to measured 24h CCr for most elderly people studied. Accuracy of prediction did not appear to be influenced by diuretic use, magnitude of measured CCr, nor frailty. However, CCr prediction using any of the 12 equations was less reliable, particularly when equations were employed which relied only upon the SCr level. While diuretic use did not affect the outcome, CCr was consistently overpredicted using the equations in those with the lowest measured CCr, ie. the frail elderly, who are probably at greatest risk from adverse drug reactions.

From this study it would seem that CCr can be most accurately predicted in elderly subjects using a urine collection of less than 24 hours. Urine collections carried out overnight are probably the most accurate & convenient, although time of day has little influence on prediction accuracy. Furthermore, diuretic use, magnitude of measured CCr and frailty do not appear to compromise the accuracy of prediction. In the elderly, CCr seems to be less well

predicted using any of the classical equations, although equations into which age & bodyweight are incorporated yield the most favourable results. Using this method of prediction CCr is likely to be overestimated in those frail individuals who have a low measured CCr. Differences in body composition between fit & frail subjects are probably important in the explanation of the reduction in prediction accuracy associated with frailty. These finding have important consequences when CCr is predicted in the course of drug dosage calculations, since those who are frail would seem to be more likely to receive an inappropriate dose. This has not previously been recognised.

From the CCr study it appears that frail subjects are likely to have a reduced CCr when compared to their fit counterparts of a similar age & sex, and it would therefore seem reasonable to suppose that renal drug clearance is also reduced in frailty. In order to test this hypothesis the renal excretion of frusemide, a commonly used diuretic, was followed in fit & frail elderly people, and the efficiency of elimination compared between the two groups. Although the frusemide study was flawed with respect to sampling times, the results were taken to be orientating and qualitative.

All subjects in this study were prescribed frusemide as Frumil or frusemide BP tablets, to treat a variety of cardiovascular conditions. Therefore, no subject was fit according to the definitions of other groups, although many were fully mobile and able to live independently in the community. It was these subjects who were categorized as fit for the purposes of the present study.

Subjects taking Frumil appeared to eliminate FRU more efficiently than those taking frusemide and so two separate groups were formed according to the preparation taken. Despite the likelihood that two populations were present, patterns observed between fit & frail groups were similar. Frail subjects tended to have an increased elimination t1/2 and reduced serum & renal clearance when compared to their fit counterparts, although it was not clear whether this trend was due to the reduced CCr also observed in the frail group or due to frailty per se. In an attempt to separate out the influences of age, CCr and frailty, fit & frail subjects taking Frumil were age-matched and the groups again compared. When no significant difference was seen for age or CCr between the two groups, frail subjects still appeared to eliminate FRU less efficiently although this observation was only of borderline significance. However, it appeared that some subjects with a low CCr excreted FRU rapidly, suggesting that in some cases nonrenal routes of elimination were important.

These results suggest that while frailty may be responsible for a small reduction in FRU elimination rate this is probably of only limited clinical significance. More important is the additional decrement in CCr which seems to be contributed by the frail state, which could be due to differences in activity, posture and renal blood flow between the two groups.

Unfortunately hepatic function cannot be measured as easily as renal function since the standard liver function tests are more a measure of hepatic damage. Therefore, since parallel studies could not be run for hepatic as renal

function, it was instead necessary to monitor the elimination of a model drug whose clearance is accomplished solely by hepatic metabolism. Paracetamol, a widely used and well tolerated analgesic, was selected as the model drug since it is known to be extensively metabolised, with only 5% of the therapeutic dose excreted unchanged in urine.

Paracetamol $t_{1/2}$ & clearance were measured and compared between groups of fit & frail subjects, and it was found that both kinetic parameters were consistently altered in frailty in such a way as to suggest impaired PAR metabolism. This observation remained when subjects were age-matched. Possible explanations for these results could include a reduction in liver perfusion or hepatic enzyme activity occurring in frailty, which could be the result of differences in diet, activity or posture. Although the exact mechanisms by which the reduction in hepatic metabolism occurred in frail subjects could not be elucidated from this study, it seems likely that reduced clearance of the model drug reflects a real difference in hepatic function between the fit & frail elderly.

Digoxin elimination has been widely investigated in elderly people since its use is associated with increased morbidity & mortality in this age group. While many studies have attempted to quantify the importance of a number of factors known to influence serum DIG concentration at steady state, a high degree of unexplained variance has rendered any resulting equations unworkable in clinical practice. Digoxin clearance was therefore compared in fit & frail subjects in order to determine whether frailty could contribute to this unexplained variance.

Fit & frail groups were comparable in age & bodyweight, but a significant difference was seen between them for CCr and DIG clearance. When the effects of renal function and frailty were separated by matching fit & frail subjects for CCr, the difference in DIG clearance between the groups was lost. This suggested that whilst frailty appeared to contribute little, if any, influence to [SDIG] for a given dosage, the reduction in CCr associated with frailty could explain a degree of the unexplained variance observed in other studies.

The detection of physiological differences between fit & frail groups defined in this thesis suggests that at least two populations of elderly people do in fact exist. While the definitions set down for the purposes of the studies differed from those used by previous groups, some constancy lay between them since the very fit subjects used in the CCr study had similar renal function to the fit subjects used in ensuing studies.

The question of why the frail elderly should handle some drugs less efficiently than their fit counterparts is not easy to answer, and little work to date has addressed this problem.

It is likely that differences in activity & posture between the two groups, with subsequent changes in blood flow patterns, may in part be responsible for some of the changes seen. However, few frail subjects appear to revert to the fit state even after hospital admission for mobilization & rehabilitation and so it was not possible to determine prospectively whether an increase in fitness & activity is associated with improved drug handling in particular patients used as their own controls. This would

be suitable material for further study although numbers would be limited by the low incidence of recovery observed during the course of these studies.
Differences in muscle mass & overall body composition are probably also important in the explanation of the changes associated with frailty.
Another important factor may be a difference in the extent of drug absorption which could occur between the groups, and again this has been poorly investigated to date. However, in both the frusemide & digoxin studies percent dose recovered in urine was similar between fit & frail groups which suggests that extent of absorption differs relatively little between them.

The importance of these findings may be seen in the light of drug dosage calculations which are at times necessary when potentially toxic drugs are prescribed whose therapeutic window is narrow. While numerous nomograms & equations exist to facilitate the selection of a correct therapeutic dosage which is unlikely to produce toxic side effects, none appear to take into account the physical condition of the patient beyond their renal or hepatic function, which is in itself often unknown. Results from this study suggest that CCr may be lower than expected for a given age & weight in frail elderly subjects, and this can lead to obvious discrepancies in drug dosage calculations. In addition, hepatic drug clearance, at least in drugs whose metabolism is via conjugation with sulphate or glucuronide moieties, may also be impaired in the frail elderly when compared to their fit counterparts.

The well documented excess of adverse drug reactions

observed in people over retirement age is undoubtedly due in part to the increased consumption of prescribed medication observed in this age group. However, it is possible that the incidence of such reactions could be reduced if the physical abilities of patients were taken into account when drugs were prescribed in addition to the normal reduction in renal and hepatic drug clearance known to occur in old age. If drug dosage levels were further reduced in those with the appearance of frailty it is possible that dose-related drug reactions could be reduced in this group of elderly people who are at greatest risk of their occurrence.

One another's light

It's hard to guess what brought me here,
Away from where I've hardly ever been and now
Am never likely to go again.

Faces are lost, and places passed
At which I could have stopped
And stopping, been glad enough.

Some faces left a mark;
And I on them might have wrought
Some kind of charm or spell
To make their futures work,

But it's hard to guess
How one thing on another
Works an influence.
We pass-
And lit briefly by one another's light
Think the way we go is right.

By Brian Patten
from "Vanishing Tricks"

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A P P E N D I C E S

Appendix A1

DEFINITIONS

FRAIL Unable to perform activities of daily living. Help required to live alone, or else live in Part III, residential or nursing home, or hospital. Unable to walk far unaided, and rarely able to go outside. Usually on regular medication, often for a number of conditions, which adversely affect quality of life.

FIT Able to perform activities of daily living unaided & safely live independently without support. May be on regular medication, but the condition for which it is prescribed is well controlled, and does not affect quality of life.

VERY FIT Able to perform all activities of daily living unaided & safely live independently without support. No abnormal pathology detectable on clinical examination & laboratory investigation. Occasional over-the-counter preparations may be taken, but no regular prescribed medication.
This definition is used in the CCr study only.

Appendix A2

MOBILITY SCORE

1 - Fully Mobile	Free walking both inside & outside the home for a good distance without effort, possible limitations only on longer distances.
2 - Limited Mobility	Free walking within the home or ward but limited mobility outdoors. At times housebound. Able to manage stairs.
3 - Restricted Mobility	Sits for most of the day. Only able to walk short distances to the toilet & rooms on the same level. Unable to manage stairs.
4 - Chairbound	Able to sit upright in chair or wheelchair. Requires help to transfer, unable to walk more than a few steps.
5 - Bedbound	Restricted to bed for the majority of time & unable to weight bear. May leave bed for short periods eg. toileting, but unable to sit upright. Requires help with feeding & needs to be turned in bed.

Appendix B1

Coat-A-Count® DIGOXIN

is a solid-phase ^{125}I radioimmunoassay designed for the quantitative measurement of digoxin in serum. It is intended strictly for *in vitro* diagnostic use as an aid in monitoring the therapeutic administration of this cardioglycoside.

Catalog Numbers: **TKDI1** (100 tubes) **TKDI2** (200 tubes) **TKDI5** (500 tubes) **TKDIX** (1000 tubes).

The 100-tube kit contains not more than 3 microcuries (111 kilobecquerels) of radioactive ^{125}I-digoxin; the 200-tube kit contains not more than 6 microcuries (222 kilobecquerels); the 500-tube kit contains not more than 15 microcuries (555 kilobecquerels), and the 1000-tube kit contains not more than 30 microcuries (1110 kilobecquerels).

Introduction

The Coat-A-Count procedure is a solid-phase radioimmunoassay, wherein ^{125}I-labeled digoxin competes for a fixed time with digoxin in the patient sample for antibody sites. The antibody being immobilized to the wall of a polypropylene tube, decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled digoxin. Counting the tube in a gamma counter then yields a number, which converts by way of a calibration curve to a measure of the digoxin present in the patient sample.

Procedure There is only one reagent to dispense, and a single one-hour incubation. All components are supplied ready to use. No centrifuge is required. Sample and tracer additions can be handled simultaneously, if desired, with the help of an automatic pipetter-diluter. The simplicity of the Coat-A-Count procedure recommends it for high-volume testing.

Separation The coated-tube methodology offers significant advantages in reliability, as well as speed and convenience, since the tubes can be vigorously decanted, without loss of antibody-bound material. This results in a clean separation of bound from free, with negligible nonspecific binding.

Data Reduction Conventional RIA techniques of calculation and quality control are applicable. The assay has been optimized for linearity in a logit-log representation throughout the range of its calibrators. Moreover, the computation can be simplified by omitting the correction for nonspecific binding, without compromising results or quality control.

Calibration The kit is equipped with human serum-based standards having digoxin values ranging from 0.5 to 8 ng/ml (0.6 -10.2 nmol/l). The calibrators are supplied in liquid form, ready to use.

Counts The tracer has a high specific activity, with total counts in the order of 50,000 cpm at iodination. Maximum binding is approximately 50%, minimizing the counting time required for adequate precision.

Accuracy Extensive recovery experiments have shown that the assay is accurate over a broad spectrum of digoxin values. Its accuracy has been further verified in patient comparison studies against a well-established, double-antibody digoxin radioimmunoassay.

Specificity The antiserum is highly specific for digoxin, with very low crossreactivity to other compounds that might be present in patient samples. Neither protein, lipemia, bilirubin nor hemolysis has any effect on the assay.

Sensitivity The procedure can detect as little as 0.1 ng/ml. CVs are low and uniform, and no end of run effect has been observed in assays involving as many as 300 tubes.

Materials Supplied—Initial Preparation

■ *Precautions:* Before opening the kit, review the paragraphs on safety, printed on the inside front cover as they relate to the safe handling and disposal of reagents containing radioactivity, human serum-derived material and sodium azide.

Catalog Number	Coated Tubes Supplied	Vials of Tracer Supplied	Sets of Calibrators Supplied
TKDI1	100	1	1
TKDI2	200	2	1
TKDI5	500	5	2
TKDIX	1000	10	3

1 DIGOXIN ANTIBODY-COATED TUBES **TDI1**

100 (200,* 500,† 1000‡) polypropylene tubes coated with antibodies to digoxin and packaged in zip-lock bags. Store refrigerated and protected from moisture, carefully resealing the bags after opening: stable at 2-8°C for at least one year from the date of manufacture. *Color:* orange.

2 [^{125}I] DIGOXIN **TDI2**

One vial (two vials,* five vials,† ten vials‡) of iodinated digoxin supplied in liquid form, ready to use. Each vial contains 105 ml. Store refrigerated: stable at 2-8°C for at least 30 days after opening, or until the expiration date marked on the vial.

3 DIGOXIN CALIBRATORS **DIC3-8**

One set (two sets,† three sets‡) of six vials, labeled A through F, of digoxin calibrators supplied in liquid form, ready to use. The zero calibrator A contains 5 ml, and each of the remaining calibrators B through F contains 2 ml. Store refrigerated: stable at 2-8°C for at least 30 days after reconstitution.

The calibrators contain respectively 0, 0.5, 1, 2, 4 and 8 nanograms of digoxin per milliliter (ng/ml) in processed human serum; equivalently: 0, 0.6, 1.3, 2.6, 5.1 and 10.2 nanomoles per liter (nmol/l). Intermediate calibration points can be obtained by mixing the calibrators in suitable proportions. The life of the calibrators may be extended by freezing. Aliquot if necessary to avoid repeated freezing and thawing.

*Pertains to the 200-tube TKDI2 kit.
†Pertains to the 500-tube TKDI5 kit.
‡Pertains to the 1000-tube TKDIX kit

Materials Required But Not Provided

- Gamma counter—compatible with standard 12×75mm tubes
- Vortex mixer

Radioimmunoassay:

- Plain 12×75mm polypropylene tubes—for use as NSB tubes, available from DPC
- Micropipets: 100 µl and 1000 µl

  For the 1.0 ml reagent addition, a reliable repeating dispenser (Nichiryo or equivalent) is also suitable. With the help of an automatic pipetter-diluter, sample and reagent additions may be handled simultaneously.

- Incubation bath capable of maintaining 37°C—neither an oven nor a heat block is suitable.
- Foam decanting rack—available from DPC

A tri-level, human serum-based immunoassay control, containing digoxin as one of over 25 assayed constituents, is available from DPC (catalog number: CON6).

Specimen Collection

The patient need not be fasting, and no special preparations are necessary. Collect blood by venipuncture into plain tubes, and separate the serum from the cells. The time of collection should be noted. The procedure calls for 100µl of serum per tube.

The samples may be stored under refrigeration for seven days, or for up to two months frozen at −20°C. Before assay, allow the samples to come to room temperature and mix by *gentle* swirling or inversion. Aliquot, if necessary, to avoid repeated thawing and freezing. Do not attempt to thaw frozen specimens by heating them in a water bath.

Radioimmunoassay Procedure

All components must be at normal room temperature before use.

1 **Plain Tubes:** Label four plain (uncoated) 12×75mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

Because nonspecific binding in the Coat-A-Count procedure is characteristically low, the NSB tubes may be safely omitted without compromising accuracy or quality control.

Coated Tubes: Label twelve Digoxin Antibody-Coated Tubes A (maximum binding) and B through F in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and patient samples.

Calibrators	ng/ml	nmol/l
A(MB)	0	0
B	0.5	0.6
C	1	1.3
D	2	2.6
E	4	5.1
F	8	10.2

2 Pipet **100µl** of the zero calibrator A into the NSB and A tubes, and **100 µl** of each remaining calibrator, control and patient sample into the tubes prepared. **Pipet directly to the bottom.**

3 Add **1.0 ml** of [^{125}I] Digoxin to every tube. Vortex.

Laboratories equipped with a reliable pipetter-diluter may handle steps 2 and 3 simultaneously. No more than ten minutes should elapse during the dispensing of the tracer. Set the T tubes aside for counting (at step 6); they require no further processing.

4 Incubate for **60 minutes at 37°C**.

Use a waterbath; neither an oven nor a heat block is suitable. The incubation may be shortened to as little as 30 minutes (at 37°C), at the expense of slightly less binding.

5 Decant thoroughly.

Removing all visible moisture will greatly enhance precision. Using a foam decanting rack, decant the contents of all tubes (except the T tubes) and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbent paper to shake off all residual droplets.

6 Count for 1 minute in a gamma counter.

Alternate "STAT" Procedure

When circumstances require fast turnaround, the following "stat" procedure may be used. The assay does not reach equilibrium under the conditions described below, and thus the timing must be meticulously controlled. The "stat" procedure should be relied on only for rough answers and only for one or two patient samples (plus controls). Careful selection of controls with digoxin concentrations above and below the region of interest will enhance the reliability of the results.

1 **Coated Tubes:** Label six Digoxin Antibody-Coated Tubes A (maximum binding), B and E. Label additional tubes in duplicate for the patient sample(s) and controls.

Calibrator	ng/ml	nmol/l
A(MB)	0	0
B	0.5	0.6
E	4	5.1

2 Pipet **100µl** of the calibrators, patient sample(s) and controls into the tubes prepared. **Pipet directly to the bottom.**

3 Add **1.0 ml** of Buffered [^{125}I] Digoxin to every tube. Vortex.

4 Incubate for **15 minutes at 50°C**.

Use a waterbath; neither an oven nor a heat block is suitable.

5 Decant thoroughly, and count for 1 minute in a gamma counter.

Calculation of Results

To calculate digoxin concentrations from a logit-log representation of the calibration curve, first calculate for each pair of tubes the average NSB-corrected counts per minute:

Net Counts = Average CPM *minus* Average NSB CPM

Then determine the binding of each pair of tubes as a percent of maximum binding (MB), with the NSB-corrected counts of the A tubes taken as 100%:

$$\text{Percent Bound} = \frac{\text{Net Counts}}{\text{Net MB Counts}} \times 100$$

The calculation can be simplified by omitting the correction for nonspecific binding; samples within range of the calibrators yield virtually the same results when Percent Bound is calculated directly from Average CPM.

Using the logit-log graph paper provided with the kit, plot Percent Bound on the vertical axis against Concentration on the horizontal axis for each of the calibrators B through F, and draw a straight line approximating the path of these five points. Digoxin concentrations for the unknowns may then be estimated from the line by interpolation.

Although other approaches are acceptable, data reduction by the logit-log method just described has certain advantages in this context – for example, in allowing easier recognition of deviant calibration points – since the Coat-A-Count Digoxin procedure has been optimized for linearity in that representation.

Example: The figures tabulated below are for illustration only and should not be used to calculate results from another assay.

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	Digoxin ng/ml
T	48,851 48,991	48,921			
NSB	184 186	185	0		
A(MB)	21,751 21,919	21,835	21,650	100.0%	0
B	17,475 17,455	17,465	17,280	79.8%	0.5
C	15,102 15,038	15,070	14,885	68.8%	1.0
D	10,903 10,861	10,882	10,697	49.4%	2.0
E	7,185 6,895	7,040	6,855	31.7%	4.0
F	4,354 4,464	4,409	4,224	19.5%	8.0
Unknowns:					
X1	15,610 16,076	15,843	15,658	72.3%	0.8
X2	12,286 12,202	12,244	12,059	55.7%	1.5
X3	7,557 7,793	7,675	7,490	34.6%	3.6

Quality Control Parameters: T = 48,921 cpm. %NSB = 0.4%. %MB = 44.3%.
20% Intercept = 7.5 ng/ml. 50% Intercept = 2.0 ng/ml. 80% Intercept = 0.5 ng/ml.

Quality Control

Record Keeping: It is good laboratory practice to record for each assay the lot numbers and reconstitution dates of the components used.

Sample Handling: The instructions for handling and storing patient samples and components should be carefully observed. Dilute high patient serum samples with the kit's zero calibrator prior to assay. All samples, including the calibrators and controls, should be assayed in duplicate. It is good laboratory practice to use a *disposable-tip* micropipet, changing the tip between samples, in order to avoid carry-over contamination. Pairs of control tubes may be spaced throughout the assay to help verify the absence of significant drift. Inspect the results for agreement within tube pairs, and take care to avoid carry-over from sample to sample.

Controls: Controls or serum pools with low, intermediate and high digoxin concentrations should routinely be assayed as unknowns, and the results charted from day to day as described in J.O. Westgard et al, "A multi-rule chart for quality control" *Clinical Chemistry 27* (1981) 493-501. See also *Scandinavian Journal of Clinical and Laboratory Investigation 44* (1984) Suppl 171 and 172. Repeat samples are a valuable additional tool for monitoring interassay precision.

Data Reduction: It is good practice to construct a graph of the calibration curve as a visual check on the appropriateness of the transformation used, even where the calculation of results is handled by computer. See further S.E. Davis et al, "Radioimmunoassay data processing with a small programmable calculator" *Journal of Immunoassay 1* (1980) 15-25; and R.A. Dudley et al, "Guidelines for immunoassay data reduction" *Clinical Chemistry 31* (1985) 1264-71.

Q. C. Parameters: We recommend keeping track of these performance measures:
- T = Total Counts (as counts per minute)
- %NSB = 100 × (Average NSB Counts ÷ Total Counts)
- %MB = 100 × ((Average MB Counts *minus* Average NSB Counts) ÷ Total Counts)

And the 20, 50 and 80 percent "intercepts," where
- 20% = Digoxin Concentration at 20 Percent Bound, etc.

Performance Characteristics

In the sections below, digoxin results are expressed as nanograms of digoxin per milliliter (ng/ml). To convert to nanomoles per liter (nmol/l), multiply by 1.281:

ng/ml × 1.281 = nmol/l

Sensitivity

Forty zero calibrator (maximum binding) tubes were processed in a single assay, along with a set of non-zero calibrators and controls. Mean and standard deviation were calculated for the counts-per-minute of the forty zero tubes. Then, from a standard curve prepared by the logit-log technique and using this mean as the zero point, the apparent digoxin concentration was determined at increasing standard deviations from the mean.

Mean ± SD of 40 MB tubes	Mean *minus*	% B/B_0	Apparent Concentration	Approximate Sensitivity
18,995 ± 268	2SD	97.1%	0.04	0.1 ng/ml
	3SD	95.7%	0.06	
	4SD	94.3%	0.08	

The detection limit (or "least detectable dose") of an assay is commonly defined as the apparent concentration two standard deviations below the counts at maximum binding or else as the concentration at 95% B/B_0. By the more conservative definition, the Coat-A-Count Digoxin assay has a detection limit of approximately 0.1 ng/ml.

Kinetics

To determine the effect of employing incubation times other than 1 hour at 37°C or 15 minutes at 50°C, as specified on pages 3 and 4, respectively, assays were set up in parallel, using incubations of 30, 60, 90 and 120 minutes at 37°C and 10, 15 and 20 minutes at 50°C. Various quality control performance measures were monitored, including: nonspecific binding and maximum binding (percent of total counts); the correlation coefficient (rho) of the logit-log line; the assay CV, based on the binding of the replicates; the 20, 50 and 80 percent intercepts (ng/ml); and the binding of the calibrators (% B/B_0). In addition, several samples were assayed as unknowns in each of the assays.

Parameter	30 min 37°	**60 min 37°**	90 min 37°	120 min 37°	10 min 50°	**15 min 50°**	20 min 50°
Total Counts	46,734 cpm	**43,046 cpm**	44,256 cpm	42,818 cpm	44,001 cpm	**43,715 cpm**	42,868 cpm
% NSB	1.3%	**1.2%**	1.2%	0.7%	0.5%	**0.5%**	0.4%
% MB	27%	**38%**	47%	50%	22%	**28%**	28%
rho	−0.997	**−0.997**	−0.999	−0.998	−0.996	**−0.998**	−0.995
Assay CV	1.7%	**1.7%**	1.8%	1.8%	2.9%	**1.9%**	1.8%
Intercepts:							
20% B/B_0	8.8 ng/ml	**7.3 ng/ml**	6.4 ng/ml	6.1 ng/ml	11.4 ng/ml	**10.4 ng/ml**	10.0 ng/ml
50% B/B_0	2.4	**2.0**	1.8	1.6	2.7	**2.3**	2.3
80% B/B_0	0.7	**0.6**	0.5	0.5	0.6	**0.5**	0.6
Calibrators:							
B −0.5 ng/ml	84%	**82%**	81%	78%	83%	**80%**	79%
C −1.0	73%	**70%**	66%	63%	72%	**70%**	72%
D −2.0	57%	**51%**	48%	46%	58%	**54%**	55%
E −4.0	36%	**32%**	29%	28%	41%	**38%**	37%
F −8.0	22%	**19%**	17%	16%	25%	**24%**	23%
Samples:							
1	0.6 ng/ml	**0.6 ng/ml**	0.4 ng/ml	0.5 ng/ml	0.5 ng/ml	**0.5 ng/ml**	0.5 ng/ml
2	0.9	**0.8**	0.8	0.9	0.9	**0.8**	0.8
3	0.9	**1.0**	1.0	0.9	0.8	**1.0**	0.7
4	1.8	**1.7**	1.7	1.6	1.9	**1.8**	1.6
5	1.8	**1.9**	2.0	1.8	1.7	**1.8**	1.7
6	1.8	**2.0**	1.9	1.9	1.6	**1.8**	1.8
7	2.5	**2.7**	2.7	2.5	2.3	**2.4**	2.5
8	2.7	**2.9**	3.1	2.9	2.5	**2.6**	2.7
9	3.1	**3.3**	3.2	3.3	2.8	**3.1**	3.0
10	3.6	**4.0**	3.8	3.5	3.6	**3.6**	3.9
Mean:	2.0 ng/ml	**2.1 ng/ml**	2.1 ng/ml	2.0 ng/ml	1.9 ng/ml	**1.9 ng/ml**	1.9 ng/ml

Based on these and similar results, one hour at 37°C was chosen as the Basic Procedure, and fifteen minutes at 50°C was chosen as the "STAT" Procedure.

Precision

The reliability of Diagnostic Products Corporation's Coat-A-Count Digoxin procedure was assessed by examining its reproducibility on samples selected to represent a range of digoxin levels.

Intraassay: Statistics were calculated for each of three samples from the results of 20 pairs of tubes in a single assay.

Sample	Mean	SD	CV
1	0.7	0.05	7.1%
2	2.5	0.10	4.0%
3	5.9	0.20	3.4%

Interassay: Statistics were calculated for each of three samples from the results of pairs of tubes in 10 different assays.

Sample	Mean	SD	CV
1	0.7	0.056	8.0%
2	2.1	0.12	5.7%
3	5.5	0.22	4.0%

Drift

To determine whether there is any position effect due to delays in the addition of reagents or decanting, pairs of tubes were spaced throughout a long assay for each of three samples. The results show no significant position (or "end of run") effect even in assays of as many as 300 tubes.

Sample	1st Pair of Tubes	Tubes Intervening	2nd Pair of Tubes	Tubes Intervening	3rd Pair of Tubes
1	0.8	138	0.9	170	0.9
2	2.5	138	2.4	170	2.5
3	5.4	138	5.5	170	5.3

Effect of Lipemia

The kit's high calibrator (8 ng/ml) was serially diluted with a lipemic serum pool. This causes the degree of lipemia to increase as the digoxin concentration (due to the calibrator) decreases. These dilutions were assayed along with the unspiked lipemic pool. The results show good recoveries even in the presence of severe lipemia.

Dilution of 8 ng/ml	O Observed	E Expected	% O/E Recovery
unspiked	0.2		
16 in 32	4.3	4.1	105%
8 in 32	2.0	2.2	91%
4 in 32	1.0	1.2	83%
2 in 32	0.7	0.7	100%
1 in 32	0.4	0.4	100%

Protein Effect

To simulate various protein concentrations, experiments were performed in which 6.0 ml aliquots of a human serum pool were freeze-dried and then reconstituted with various volumes of water. Each reconstituted aliquot was then assayed by the Coat-A-Count Digoxin procedure. Note that aliquots reconstituted with half the original volume represent an extremely high protein concentration, in the order of 14 gm/dl. These results indicate that even wide variations in protein have virtually no effect on the Coat-A-Count Digoxin assay.

Experiment	Reconstitution		Protein Concentration	O Observed	E Expected	% O/E Recovery
1	3.0 ml	2.00×	~14.0 gm/dl	4.6	4.6	100%
	6.0	1.00×	~ 7.0	2.3	—	(100%)
	12.0	0.50×	~ 3.5	1.1	1.2	92%
2	3.0 ml	2.00×	~14.0 gm/dl	11.3	12.0	94%
	6.0	1.00×	~ 7.0	6.0	—	(100%)
	12.0	0.50×	~ 3.5	2.9	3.0	100%
3	3.0 ml	2.00×	~14.0 gm/dl	4.0	4.0	100%
	6.0	1.00×	~ 7.0	2.0	—	(100%)
	12.0	0.50×	~ 3.5	1.0	1.0	100%
4	3.0 ml	2.00×	~14.0 gm/dl	11.0	11.2	98%
	6.0	1.00×	~ 7.0	5.6	—	(100%)
	12.0	0.50×	~ 3.5	3.0	2.8	107%

Specificity

The digoxin antiserum is highly specific for digoxin, with an extremely low crossreactivity to other naturally occurring steroids or therapeutic drugs that may be present in patient samples.

Compound	ng/ml Added	Apparent Concentration – ng/ml	Crossreactivity
Deslanoside C	10	5.4	0.54
	5	2.6	0.52
	1	0.5	0.50
Lanatoside	100	33.3	0.33
	10	3.6	0.36
	1	0.5	0.50
Digitoxin	1,000	3.7	0.0037
	500	2.1	0.0042
Dihydrodigoxin	5	0.06	0.012
	1	0	–
Cortisol	100,000	0.6	0.000006
	4,000	0.05	0.000013

From the table it is clear that the Coat-A-Count Digoxin procedure is not suitable for use on patients receiving either Deslanoside C or Lanatoside, two compounds with molecular structures similar to that of digoxin.

Effect of Bilirubin and Hemolysis

To simulate severe icterus, three serum samples were spiked with 20 mg/dl of bilirubin. In another experiment, to simulate mild, moderate and severe hemolysis, three serum samples were spiked with 10, 15 and 30 µl/ml of packed red blood cells. All samples were assayed both spiked and unspiked by the Coat-A-Count Digoxin procedure with the following results.

Sample	Unspiked	Bilirubin 20 mg/dl
1	0.9	1.2
2	2.5	2.5
3	6.1	5.8

Sample	Unspiked	Packed Red Blood Cells µl/ml 10	15	30
1	0.9	0.9	0.9	1.0
2	2.5	2.5	2.5	2.6
3	6.1	5.9	5.7	5.6

The results show that neither severe icterus (bilirubin up to 20 mg/dl) nor gross hemolysis has any effect on the Coat-A-Count Digoxin procedure.

Matrix Effects

The Coat-A-Count Digoxin calibrators are human serum based. To determine the effect on the assay of preparing the calibrators in other matrix materials, several digoxin-free materials were assayed by the Coat-A-Count procedure. Note that all results are below the detection limit of the assay.

Matrix	% B/B_0	Apparent Digoxin Concentration
Normal Human Serum Charcoal-Absorbed, #393	101.0%	0.00 ng/ml
Normal Human Serum Charcoal-Absorbed, #059	97.2%	0.04 ng/ml
Normal Human Plasma Heparinized, Charcoal-Absorbed	104.0%	–
5% Bovine Serum Albumin	100.0%	0.00 ng/ml
10% Human Serum Albumin Charcoal-Absorbed	96.5%	0.07 ng/ml

Effect of Anticoagulants

To determine whether anticoagulants interfere with the assay, blood was collected from six normal volunteers into plain, heparinized and EDTA vacutainer tubes. All samples were assayed by the Coat-A-Count Digoxin procedure, with the following results.

Sample	Serum	Heparin	EDTA
1	5.9	5.4	5.4
2	4.6	4.9	5.1
3	5.7	4.1	5.7
4	5.7	5.1	5.3
5	5.6	5.0	5.8
6	5.1	5.3	5.4
Average:	5.4	5.0	5.5

On the basis of these results it is recommended that samples be collected as serum.

Parallelism

Six patient serum samples were assayed both undiluted and diluted with the kit's zero calibrator. The observed and expected values are presented below in ng/ml.

Sample	Dilution	O Observed	E Expected	% O/E Recovery
1	8 in 8 (undiluted)	6.1		
	4 in 8	2.9	3.0	97%
	2 in 8	1.6	1.5	107%
	1 in 8	0.9	0.8	113%
2	8 in 8 (undiluted)	5.8		
	4 in 8	2.8	2.9	97%
	2 in 8	1.4	1.5	93%
	1 in 8	0.8	0.7	114%
3	8 in 8 (undiluted)	6.8		
	4 in 8	3.0	3.4	88%
	2 in 8	1.5	1.7	88%
	1 in 8	0.8	0.9	89%
4	8 in 8 (undiluted)	6.9		
	4 in 8	3.6	3.5	103%
	2 in 8	1.8	1.7	106%
	1 in 8	0.9	0.9	100%
5	8 in 8 (undiluted)	6.7		
	4 in 8	3.4	3.4	100%
	2 in 8	1.8	1.7	106%
	1 in 8	0.9	0.8	113%
6	8 in 8 (undiluted)	7.2		
	4 in 8	3.6	3.6	100%
	2 in 8	1.8	1.8	100%
	1 in 8	1.0	0.9	111%

Curve Displacement

Four samples were each assayed by the Coat-A-Count Digoxin procedure in assays which included one-to-one dilutions of the sample with each of the calibrators A through F.

Sample	O Observed	E Expected	% O/E Recovery
1 unspiked	0.0		
1 + A (0)	0.0	0.0	100%
1 + B (0.5)	0.3	0.3	100%
1 + C (1)	0.5	0.5	100%
1 + D (2)	1.0	1.0	100%
1 + E (4)	2.0	2.0	100%
1 + F (8)	3.8	4.0	95%
2 unspiked	0.4		
2 + A (0)	0.2	0.2	100%
2 + B (0.5)	0.4	0.5	80%
2 + C (1)	0.8	0.7	114%
2 + D (2)	1.2	1.2	100%
2 + E (4)	2.1	2.2	95%
2 + F (8)	4.2	4.2	100%
3 unspiked	0.8		
3 + A (0)	0.5	0.4	125%
3 + B (0.5)	0.7	0.7	100%
3 + C (1)	1.0	0.9	111%
3 + D (2)	1.4	1.4	100%
3 + E (4)	2.5	2.4	104%
3 + F (8)	4.3	4.4	98%
4 unspiked	2.8		
4 + A (0)	1.3	1.4	93%
4 + B (0.5)	1.7	1.7	100%
4 + C (1)	2.0	1.9	105%
4 + D (2)	2.4	2.4	100%
4 + E (4)	3.4	3.4	100%
4 + F (8)	5.2	5.4	96%

Comparison with a Reference Method

With DPC's well-established Double Antibody Digoxin radioimmunoassay serving as the reference method, 100 patient samples were simultaneously assayed by the solid-phase Coat-A-Count kit and by the Double Antibody kit. Linear regression analysis of the results yielded the following relationship.

(Coat-A-Count) = 0.97 (Double Antibody) − 0.05 ng/ml
r = 0.988 SEE = 0.16 n = 100

Clinical Applications

Immunoassay has proved itself a particularly useful aid in determining drug overdoses in patients treated with cardiac glycosides. The technique is also useful (a) in clarifying situations where a patient's symptoms might be due either to intrinsic heart disease or to digitalis intoxication; (b) where there is doubt concerning the type of digitalis preparation the patient is taking – in this case, digitoxin immunoassay is also necessary; (c) for measuring the digoxin ingestion of patients with an inadequate history of previous dosage; (d) in documenting cases of underdigitalization as well as digitalis (digoxin) excess; (e) in monitoring the toxic response in patients with myocardial disease associated with hypokalemia, hypomagnesemia, hypercalcemia, hypoxia and alkalosis, which are particularly sensitive to digitalis; and (f) in preventing overdigitalization, particularly in patients whose renal function is deteriorating or for whom an increased digoxin dosage is contemplated.

The high sensitivity of digoxin immunoassay is especially necessary in view of the small differences and occasional overlap that exist between therapeutic and toxic levels of circulating digoxin. (Intoxication is defined in terms of arrhythmias and disturbances of cardiac conduction due to the drug's presence.) Smith et al, in 1969, reported serum digoxin concentrations of 0.8 - 2.4 ng/ml (1.0 - 3.1 nmol/l) in nontoxic patients, and 2.1 - 8.7 ng/ml (2.7 - 11.1 nmol/l) in toxic patients, based on data taken six hours post-dose in patients with normal renal function.[8] More recent clinical investigations have confirmed the association of toxicity with serum levels above 2 ng/ml. However, sole reliance on the digoxin concentration for the determination of digitalis toxicity is not warranted and must be supplemented with additional clinical and electrocardiographic information.

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Technical Assistance: If questions arise concerning the Coat-A-Count Digoxin reagents, or for further advice on technique, data reduction, quality control or expected values, please contact DPC and ask for "Technical Services."
(213) 776-0180
(800) 678-6699
Telex 4720518
Fax (213) 642-0192

B 782

DPC
Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045

Protocol Date:
November 5, 1986

Appendix C1

INFORMATION SHEET FOR KIDNEY FUNCTION STUDY

Dr. Parker is carrying out a project with St. Marks Road surgery and the Research Institute for Care of the Elderly, St. Martins Hospital, looking at the way in which kidneys function as the body ages. This involves collecting all urine passed during a 24 hour period. Thank you for helping us with this study.

These instructions should be followed carefully during the period of the study. If you are not sure what to do at any time, then let someone know - phone the numbers given at the bottom of this sheet.

1) When you wake up note the exact time at which you empty your bladder (pass water) first thing. Throw this specimen away, into the toilet.

2) Each and every time you pass a urine specimen after this time, for the next 24 hours, please collect it, in the container provided. ALL urine should be collected, even at night time.

3) Exactly 24 hours later, from the passing your first sample of water (into the toilet) please pass your final specimen, and save it.

4) Dr. Parker will call or telephone during the first day of the study, to check that you are having no problems with the urine collection. He will call to take a small blood sample first thing in the morning when the urine collection is completed.

5) Please record how much meat you eat during the study.

6) Dr. Parker will ask you at what time you got up, and went to bed, on the study day, and how far you walked that day.

Very many thanks.

NAME.....

DAY OF STUDY.....

TIME URINE COLLECTION STARTED.....

TIME URINE COLLECTION TO FINISH.....

MEAT EATEN.....

IN CASE OF QUERY PLEASE DO NOT HESITATE TO CONTACT SUE ELLMERS (STUDY CO-ORDINATOR) AT WORK ON BATH 835866
OR AT HOME ON BATH 834963

Appendix C2

MEASUREMENT OF 24h CREATININE CLEARANCE IN THE ELDERLY

Name : . . . . . Study No. : . . . .

Address : . . . . . Study Date : . . . .

. . . . . .

. . . . . Mobility Score : . .

. . . . . .

. . . . . Height/cm. . . . . .

Tel. No : . . . . . .

Weight/kg. . . . . .

D.O.B. : . . . . . .

Age : . . . . . SA/m2. . . . . .

G.P. : . . . . . .

Diagnoses : . . . . . .

. . . . . .

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. . . . . .

Continent or catheterised:

Drugs : . . . . . .

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. . . . . .

. . . . . .

. . . . . .

Start urine colln..... Date..... (discard urine)

Urine Samples :		Time	Vol/ml	Combined
	1)	. . . . .	. . . . .	. . . . .
	2)	. . . . .	. . . . .	. . . . .
alcohol	3)	. . . . .	. . . . .	. . . . .
	4)	. . . . .	. . . . .	. . . . .
	5)	. . . . .	. . . . .	. . . . .
tobacco	6)	. . . . .	. . . . .	. . . . .
	7)	. . . . .	. . . . .	. . . . .
	8)	. . . . .	. . . . .	. . . . .
meat	9)	. . . . .	. . . . .	. . . . .
	10)	. . . . .	. . . . .	. . . . .
	11)	. . . . .	. . . . .	. . . . .
	12)	. . . . .	. . . . .	. . . . .
	13)	. . . . .	. . . . .	. . . . .
	14)	. . . . .	. . . . .	. . . . .

TOTAL URINE VOL : . . . . . IN . . . . .HRS

Comments. . . . . .

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Appendix C3

MEASUREMENT OF SINGLE 24h CCR IN THE ELDERLY

Name : . . . . . Study No. : . . . .

Address : . . . . . Study Date : . . . .
. . . . . .
. . . . . Mobility Score : . .
. . . . . .
Tel. No : . . . . . .

D.O.B. : . . . . . .
Age : . . . . . .
G.P. : . . . . . .

Height/cm : . . . . . SA/m2 : . . . . .
Weight/kg : . . . . . .

Diagnoses : . . . . . .
. . . . . .
. . . . . .
. . . . . .
. . . . . .

Continent or catheterised:

Drugs : . . . . . .
. . . . . .
. . . . . .
. . . . . .
. . . . . .
. . . . . .

BM-Stix

Time Collection started : . . . . . pH
protein
First urine specimen discarded Y/N glucose

Total volume urine . . . . . alcohol
. . . . . tobacco smoker
. . . . . .
. . . . . .

Total collection period . . . . . .hours

Time of blood sample . . . . . .

Meat Eaten:

R.I.C.E.
St. Martins Hospital,
Bath. (Tel : 835866)

Comments. . . . . .
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Appendix C4 Demographic Details & Medications of Subjects in CREATININE Study

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F07CC	F / 80	64/163	Y / 1	no	9/wk	cramps	oxerutins
F09CC	F / 66	63/160	Y / 1	no	occ.	nil	nil
F15CE	F / 76	66/159	Y / 1	no	no	nil	nil
F18CC	F / 65	61/155	Y / 1	no	no	nil	nil
F19C	F / 79	62/170	Y / 1	no	no	hypothyroid	thyroxine,indomethacin prochlorperazine
F20CE	F / 84	60/156	Y / 2	ex.	7/wk	OA	nil
F21CE	F / 72	55/161	Y / 1	no	occ.	nil	nil
F23CC	F / 71	70/159	Y / 1	no	occ.	depression	dothiepin
F27CE	F / 64	74/160	Y / 1	no	occ.	nil	ferrous fumerate,vitamins homeopathic Flu tablets
F30CE	F / 60	79/159	Y / 1	no	occ.	nil	aspirin prn
F31CC	F / 73	64/160	Y / 1	ex.	occ.	hypertension	nifedipine,co-amilozide co-proxamol
F36CE	F / 69	51/161	Y / 1	no	no	nil	nil
F37C	F / 66	55/160	Y / 1	no	occ.	nil	nil
F39C	F / 76	47/152	Y / 1	no	occ.	nil	nil
F41CE	F / 60	70/166	Y / 1	no	no	nil	nil
F44CC	F / 67	69/167	Y / 1	no	occ.	hypertension	Navidrex K
F46CC	F / 67	61/161	Y / 1	no	no	OA,nephrectomy @ 15y	indomethacin, herbs
F47C	F / 70	53/156	Y / 1	16/day	no	nil	nil
F48C	F / 70	57/156	Y / 1	no	occ.	hypertension	oxprenolol, ranitidine
F49C	F / 66	72/159	Y / 1	ex.	occ.	hypertension	nicardipine, Dyazide
F50C	F / 73	65/158	Y / 1	no	occ.	OA	indomethacin, temazepam
F51CE	F / 73	78/155	Y / 1	ex.	occ.	OA, hypothyroid	ibuprofen, thyroxine bendrofluazide
F53CC	F / 67	75/159	Y / 1	no	occ.	gout	allopurinol,chlorthiazide
F55C	F / 73	61/153	Y / 1	no	occ.	nil	aspirin, diazepam
F58C	F / 75	72/160	Y / 1	no	occ.	nil	nil

Appendix C4 Demographic Details & Medications of Subjects in CREATININE Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F59C	F / 72	66/143	Y / 1	no	occ.	mild renal impairment	Burinex K, KCl Beconase
F60C	F / 63	60/164	Y / 1	no	occ.	hypertension	Tenoret, multivitamins
F62C	F / 73	77/155	Y / 1	no	occ.	nil	nil
F63C	F / 66	60/162	Y / 1	no	14/wk	migraine depression	propranolol,dothiepin Alophen
F65C	F / 75	73/175	Y / 1	no	occ.	Ca breast	tamoxifen
F66CE	F / 60	75/165	Y / 1	no	occ.	nil	nil
F68CC	F / 79	70/165	Y / 1	no	occ.	nil	aspirin
F69CC	F / 73	67/170	Y / 1	no	occ.	PA	B12 injections
F71CE	F / 64	63/160	Y / 1	no	14/wk	nil	nil
F73CC	F / 65	79/164	Y / 1	no	occ.	nil	nil
F74C	F / 70	60/155	Y / 1	no	occ.	OA	diclofenac
F75CC	F / 70	65/163	Y / 1	no	occ.	nil	nil
F76CE	F / 67	80/167	Y / 1	ex.	occ.	hypothyroid	thyroxine
F78CE	F / 66	66/169	Y / 1	no	7/wk	nil	nil
F81CE	F / 65	62/158	Y / 1	ex.	3/wk	nil	piperazine oestrone
F86CC	F / 67	56/161	Y / 1	no	occ.	nil	bisacodyl
F91CE	F / 68	56/157	Y / 1	10/day	3/wk	nil	nil
F93C	F / 66	76/167	Y / 1	ex.	21/wk	nil	betahistine
F94CC	F / 73	70/149	Y / 1	1/day	occ.	nil	nitrazepam
F96CE	F / 64	53/150	Y / 1	no	occ.	nil	nil
F97CC	F / 71	59/164	Y / 1	no	occ.	nil	nil
M08CC	M / 84	85/184	Y / 1	1/day cigar	yes mod.	nil	nil
M22CC	M / 70	90/175	Y / 1	no	occ.	nil	Rennies
M35CE	M / 71	77/179	Y / 1	ex.	occ.	nil	nil
M38C	M / 65	58/161	Y / 1	no	occ.	depression	mianserin
M43C	M / 60	93/177	Y / 1	no	yes ? excess	nil	nil

Appendix C4 Demographic Details & Medications of Subjects in CREATININE Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
M54CC	M / 62	60/166	Y / 1	no	occ.	nil	nil
M57CE	M / 68	71/166	Y / 1	8/day	7/wk	nil	nil
M61CC	M / 73	88/183	Y / 1	no	occ.	nil	nil
M64C	M / 75	68/168	Y / 1	ex.	occ.	glaucoma	pilocarpine ED
M67CE	M / 64	79/183	Y / 1	heavy-pipe	occ.	nil	nil
M72CC	M / 72	80/184	Y / 1	no	occ.	nil	nil
M77CE	M / 72	67/173	Y / 1	ex.	occ.	nil	nil
M79CE	M / 73	63/162	Y / 1	2/day	occ.	nil	nil
M80CC	M / 66	81/175	Y / 1	no	40/wk	nil	nil
M82CC	M / 66	78/172	Y / 1	no	no	nil	nil
M83CC	M / 67	81/171	Y / 1	no	20/wk	nil	nil
M84CE	M / 65	70/171	Y / 1	no	7/wk	nil	nil
M85CC	M / 67	84/180	Y / 1	no	occ.	nil	nil
M92CC	M / 68	78/171	Y / 1	yes	14/wk	nil	nil
M95CE	M / 70	66/164	Y / 1	no	1/wk	nil	nil
M98CE	M / 64	74/172	Y / 1	no	occ.	nil	nil
M99CC	M / 65	66/168	Y / 1	no	occ.	nil	nil
M100CE	M / 64	70/164	Y / 1	no	9/wk	nil	nil
F08C	F / 87	50/142	N / 4	no	no	Parkinsonism diverticulitis	temazepam
F09C	F / 78	65/160	N / 3	no	no	CVA	none
F12C	F / 74	71/170	N / 4	no	no	anaemia, leg ulcer hypertension	Neonaclex K,oxprenolol dihydrocodeine,hydralazine temazpem,iron sulphate
F15C	F / 82	65/150	N / 4	no	no	OA, schizophrenia (burnt out)	procyclidine,fluphanazine ispagula husk
F16C	F / 78	41/150	N / 4	no	no	NIDDM, catheterised CVA & L hemiplegia ex-alcoholic	tolbutamide,terodiline

Appendix C4 Demographic Details & Medications of Subjects in CREATININE Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F17C	F / 79	83/178	N / 4	no	no	cellulitis L leg mastectomy 1978	tamoxifen,paracetamol temazepam
F18C	F / 84	45/163	N / 3	no	no	mentally subnormal pressure sore	thioridazine,Milpar
F21C	F / 88	50/152	N / 4	no	no	diverticulitis CVA, OA	none
F22C	F / 73	63/157	N / 4	no	no	CVA & R hemiplegia MI 1986	paracetamol,lactulose
F24C	F / 77	92/157	N / 4	no	no	CVA & R hemiplegia hypertension, OA	Trasidrex,paracetamol aspirin
F25C	F / 79	44/152	N / 4	no	no	spasticity of legs ? MS, catheterised	paracetamol,temazepam prochlorperazine
F28C	F / 86	50/163	N / 3	occ.	occ.	RA, constipation weight loss	mianserin,aspirin ispaghula husk
F29C	F / 83	45/150	N / 3	no	no	?acoustic neuroma	none
F30C	F / 83	55/160	N / 3	no	no	hypertension, IHD	lactulose,senna
F31C	F / 84		N / 4	no	no	diarrhoea, falls	ampicillin
F34C	F / 79	83/178	N / 3	no	no	mastectomy 1978	tamoxifen,paracetamol
F37C	F / 73	63/157	N / 3	no	no	CVA & R hemiplegia	paracetamol,lactulose
F39C	F / 88		N / 4	no	no	hypotension # R arm, falls	ranitidine,paracetamol fludrocortisone,temazepam
F41C	F / 81	50/163	N / 3	no	no	fast AF, CCF	digoxin,co-amilofruse isosorbide DN,temazepam
F42C	F / 88	48/152	N / 4	no	no	tachycardia, TIA's temporal arteritis CVA & L hemiplegia	digoxin,co-amilofruse aspirin,paracetamol prednisolone
F44C	F / 89	43/157	N / 3	no	no	NIDDM, below knee amputation	digoxin,timolol ED Milpar
F46C	F / 71	55/152	N / 3	no	no	AF, falls mitral valve disease carebral embolus '75	digoxin,co-proxamol warfarin,temazepam bendrofluazide
F02GPC	F / 78	83/152	Y / 1	no	no	hypertension bilateral THR	captopril,canesten cream
F04GPC	F / 79	55/152	Y / 1	no	no	sinusitis	none

Appendix C4 Demographic Details & Medications of Subjects in CREATININE Study (contd)

subject	sex/age	wt/ht	fit/HSc	smoker	EtOH	diagnoses	drugs
F05GPC	F / 78	51/163	Y / 1	no	no	epilepsy	carbamazepine,phenytoin inositol nicotinate
F07GPC	F / 80	62/165	Y / 1	no	no	hypothyroid, reflux	thyroxine,cinnarazine
F09GPC	F / 80	56/147	Y / 1	no	occ.	cervical spondylosis neuritis	co-proxamol,nabumetone
M02C	M / 80	71/180	N / 4	no	no	CVA & hemiplegia	thioridazine
M04C	M / 88	65/175	N / 5	ex.	occ.	MI, OA, COAD catheterised	Neonaclex K,chlormethiazole mianserin,paracetamol
M06C	M / 68	70/180	N / 3	yes heavy	yes ?	hypertension depression ex-alcoholic	allopurinol,atenolol chlormethiazole thioridazine
M07C	M / 80	80/170	N / 3	no	no	CVA, diverticulitis	ranitidine
M10C	M / 78	55/173	N / 5	no	no	IHD, CCF, anaemia	Frumil,FeSO4,paracetamol
M13C	M / 68	72/173	N / 4	no	no	IDDM, catheterised CVA & L hemiplegia	Actrapid,Monotard ibuprofen
M14C	M / 87	58/163	N / 3	no	no	asthma, OA leg ulcer	Frumil,aminophylline diazepam,paracetamol salbutamol & Becotide inh.
M19C	M / 97	50/170	N / 5	no	no	COAD	metoclopramide,ranitidine
M20C	M / 88	88/175	N / 4	ex.	7/wk	OA, COAD catheterised	Neonaclex K,paracetamol mianserin
M23C	M / 81	60/163	N / 4	no	no	CVA & hemiplegia urinary frequency	Frumil, acebutolol
M26C	M / 78	63/168	N / 4	no	occ.	CVA,hemiplegia,NIDDM	oxprenolol,paracetamol
M27C	M / 83	65/178	N / 5	N/K	N/K	AF, CCF, CVA	digoxin,ampicillin protriptyline,aspirin
M32C	M / 76	62/178	N / 4	no	no	? MI lung fibrosis	Atrovent & salbutamol nebs Becotide inh,ranitidine
M35C	M / 81	60/178	N / 4	N/K	N/K	NIDDM, glaucoma ulcer L leg	glibenclamide,co-proxamol pilocarpine ED
M43C	M / 72	86/168	N / 4	N/K	no	AF,CCF,hypertension Parkinsonism dementia, epilepsy CVA, hemiplegia	digoxin,Frumil,folic acid sodium valproate ascorbic acid,paracetamol

Appendix C4 Demographic Details & Medications of Subjects in CREATININE Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
M01GPC	M / 82	67/173	Y / 1	no	no	cardiac pacemaker peptic ulcer	warfarin,aminophylline cimetidine
M03GPC	M / 76	87/174	Y / 1	no	yes ? excess	OA, renal stone	diclofenac,quinine sulphate
M06GPC	M / 79	68/168	Y / 1	no	no	nocturia	Mucaine
M08GPC	M / 82	74/175	Y / 1	no	no	hypertension migraine	propranolol,paracetamol
M10GPC	M / 82	75/174	Y / 1	no	7/wk	DU	yeast tablets
M11GPC	M / 88	66/170	Y / 1	no	no	cholecystectomy	digoxin

wt = weight (kg)

ht = height (cm)

fit = fit Y
frail N

MSc = mobility score

EtOH = alcohol (units/week)

Appendix C5 SCr, UCr & CCr, calculated over 8 & 24 hour periods, for Subjects in CREATININE study

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F07CC	1.35	24.00	24.00	1128.0	44.4	2540		58		1.70	59
F09CC	1.25	1410	7.16	351.1	64.9	541	65		98		
		2145	7.59	404.7	43.4	933	71		108		
		0700	9.25	440.9	72.3	610	64		97		
			24.00	1196.7	57.4	2084		66		1.65	70
F18CC	1.48	24.00	24.00	983.7	83.0	1185		46		1.59	50
F23CC	1.00	1350	6.84	411.5	137.2	300	100		104		
		2235	8.75	497.6	110.6	450	95		99		
		0700	8.41	472.1	67.4	700	94		98		
			24.00	1381.2	95.3	1450		96		1.67	99
F31CC	1.08	1430	7.50	432.3	49.1	880	89		131		
		2200	7.50	399.4	39.9	1000	82		121		
		0700	9.00	226.2	60.3	375	39		57		
			24.00	1057.9	46.9	2255		68		1.68	70
F44CC	1.06	24.00	24.00	968.2	52.5	1845		63		1.76	62
F46CC	1.14	1350	6.83	612.7	102.1	600	131		170		
		2240	8.84	345.9	43.2	800	57		74		
		0640	8.00	284.0	29.9	950	52		68		
			23.67	1242.6	52.9	2350		77		1.66	80
F53CC	1.04	24.00	24.00	989.5	83.2	1190		66		1.77	65
F68CC	1.14	24.00	24.00	1087.6	60.1	1810		66		1.77	65
F69CC	1.20	1430	7.50	258.6	95.8	270	48		92		
		2315	8.75	314.1	37.6	835	50		96		
		0700	7.75	321.7	56.4	570	58		112		
			24.00	894.4	53.4	1675		52		1.78	50
F73CC	1.07	24.00	24.00	863.9	94.5	914		56		1.85	52
F75CC	1.65	1600	9.00	378.2	109.9	344	42		108		
		2208	6.13	239.1	103.1	232	39		100		
		0700	8.87	313.7	49.5	634	36		92		
			24.00	931.0	76.9	1210		39		1.70	40
F86CC	1.53	24.00	24.00	1119.5	43.7	2560		51		1.57	56
F94CC	1.62	1500	8.00	349.4	50.3	695	45		113		
		2345	8.75	331.9	85.1	390	39		98		
		0700	7.25	246.1	100.9	244	35		88		
			24.00	927.4	69.8	1329		40		1.65	42
F97CC	2.96	24.00	24.00	1190.8	65.4	1820		28		1.62	30
F15CE	1.26	24.00	24.00	753.5	38.9	1935		42		1.67	43

Appendix C5 SCr, UCr & CCr, calculated for 8 & 24h periods, for Subjects in CREATININE study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F20CE	1.37	1300	6.00	152.6	87.2	175	31		63		
		2230	9.50	426.9	82.1	520	55		112		
		0700	8.50	378.8	41.9	905	54		110		
			24.00	958.3	59.9	1600		49		1.59	53
F21CE	0.91	1329	6.48	281.5	67.0	420	80		110		
		0020	10.85	396.7	57.1	695	67		92		
		0652	6.54	277.5	45.5	610	78		107		
			23.87	955.7	55.4	1725		73		1.57	81
F27CE	1.48	1300	6.00	345.8	46.8	739	65		112		
		2400	11.00	509.9	76.7	665	52		90		
		0700	7.00	372.5	63.1	590	60		103		
			24.00	1228.2	61.6	1994		58		1.76	57
F30CE	1.05	1405	7.08	483.7	191.2	253	108		103		
		2326	9.34	579.0	83.4	694	98		93		
		0650	7.41	520.2	84.7	614	111		106		
			23.83	1582.9	101.4	1561		105		1.81	101
F36CE	1.49	1310	6.16	270.6	62.1	436	49		104		
		2230	9.34	415.1	70.2	591	50		106		
		0700	8.50	325.6	62.0	525	43		91		
			24.00	1011.3	65.2	1552		47		1.52	54
F41CE	1.23	24.00	24.00	1135.8	57.8	1965		64		1.78	62
F51CE	1.29	24.00	24.00	1116.9	60.2	1855		60		1.78	58
F66CE	1.27	24.00	24.00	1141.2	61.0	1870		62		1.83	59
F71CE	0.84	1425	7.42	318.2	92.0	346	85		106		
		2140	7.26	304.3	127.9	238	83		104		
		0700	9.32	347.9	101.1	344	74		93		
			24.00	970.4	104.6	928		80		1.65	84
F76CE	1.80	1430	7.50	433.1	76.7	565	53		115		
		2300	8.50	379.5	79.7	476	41		89		
		0700	8.00	375.7	107.3	350	43		93		
			24.00	1188.3	85.4	1391		46		1.88	42
F78CE	1.06	1437	7.61	226.1	36.1	626	47		107		
		2215	7.64	228.2	24.0	950	47		107		
		0700	8.75	210.1	34.8	604	38		86		
			24.00	664.4	30.5	2180		44		1.75	42
F81CE	1.13	1317	6.28	252.7	99.9	253	59		91		
		2206	8.82	391.9	47.3	828	66		102		
		0646	8.67	397.5	44.9	886	68		105		
			23.77	1042.1	53.0	1967		65		1.64	68
F91CE	1.03	24.00	24.00	915.5	151.3	605		62		1.54	69

Appendix C5 SCr, UCr & CCr, calculated for 8 & 24h periods, for Subjects in CREATININE study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F96CE	1.15	1530	8.50	369.8	44.6	830	63		113		
		2120	5.83	208.2	37.4	556	52		93		
		0700	9.67	353.2	75.1	470	53		95		
			24.00	931.2	50.2	1856		56		1.47	66
F19C	1.16	24.00	24.00	665.3	53.2	1250		40		1.73	40
F37C	1.02	1320	6.33	310.3	50.7	612	80		98		
		2230	9.17	488.5	49.5	986	87		106		
		0700	8.50	403.2	74.4	542	78		95		
			24.00	1202.0	56.2	2140		82		1.56	91
F39C	0.79	1405	6.09	248.4	100.6	247	86		102		
		2210	8.07	324.9	48.3	673	85		101		
		0720	9.17	356.7	57.5	620	82		98		
			23.33	930.0	60.4	1540		84		1.40	101
F47C	1.24	1352	6.87	365.2	41.9	872	71		109		
		2329	9.61	418.9	95.2	440	59		91		
		0656	7.45	374.2	97.4	384	68		105		
			23.93	1158.3	68.3	1696		65		1.51	74
F48C	1.23	24.00	24.00	945.0	66.5	1420		53		1.57	59
F49C	1.46	24.00	24.00	1036.9	60.5	1715		49		1.73	49
F50C	1.28	24.00	24.00	994.8	46.5	2140		54		1.67	56
F55C	1.27	24.00	24.00	786.0	41.4	1900		43		1.57	47
F58C	1.20	24.00	24.00	1038.8	59.4	1750		60		1.78	58
F59C	1.28	24.00	24.00	1022.3	56.5	1810		55		1.55	62
F60C	1.19	1410	7.17	283.9	76.9	369	55		95		
		2240	8.50	374.3	61.3	611	62		107		
		0640	8.25	328.9	40.6	810	56		97		
			23.92	987.1	55.1	1790		58		1.65	61
F62C	1.22	24.00	24.00	968.4	39.9	2430		55		1.76	54
F63C	1.10	1530	8.50	322.3	102.0	316	57		95		
		2330	8.00	354.9	105.0	338	67		112		
		0700	7.50	276.4	106.3	260	56		93		
			24.00	953.6	104.3	914		60		1.63	64
F65C	0.89	1535	8.59	397.3	151.1	263	87		91		
		2305	7.49	476.6	157.3	303	119		124		
		0700	7.92	355.4	70.2	506	84		88		
			24.00	1229.3	114.7	1072		96		1.87	89

Appendix C5 SCr, UCr & CCr, calculated for 8 & 24h periods, for Subjects in CREATININE study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F74C	1.44	1600	9.00	126.1	37.1	340	16		59		
		2245	6.75	229.8	82.1	280	39		144		
		0700	8.25	200.6	59.0	340	28		104		
			24.00	556.5	58.0	960		27			
F93C	1.43	24.00	24.00	808.0	28.3	2860		39		1.84	37
M99CC	1.88	1400	7.00	274.2	43.9	624	35		78		
		2245	8.75	621.8	139.4	446	63		140		
		0700	8.25	332.9	79.6	418	36		80		
			24.00	1228.9	82.6	1488		45		1.74	45
M92CC	1.44	24.00	24.00	1930.3	65.9	2930		93		1.91	84
M82CC	1.86	24.00	24.00	1590.9	94.7	1680		59		1.95	53
M85CC	1.52	24.00	24.00	1342.6	46.8	2870		61		2.05	52
M54CC	1.10	24.00	24.00	1223.1	93.4	1310		77		1.67	80
M83CC	1.40	24.00	24.00	1655.7	138.0	1200		82		1.94	73
M22CC	1.25	1240	5.67	470.5	192.0	245	111		96		
		2300	10.34	942.3	269.2	350	122		105		
		0705	8.07	680.5	247.5	275	112		97		
			24.08	2093.5	240.6	870		116		2.05	98
M80CC	1.80	24.00	24.00	1463.5	121.4	1206		56		1.98	49
M72CC	1.13	1256	5.93	445.3	55.2	806	111		119		
		2359	11.05	706.7	90.4	782	94		101		
		0710	7.19	366.4	75.4	486	75		81		
			24.17	1518.4	73.2	2074		93		2.02	80
M08CC	1.95	24.00	24.00	1720.9	66.4	2590		61		2.08	51
M61CC	1.25	24.00	24.00	1660.0	66.5	2495		92		2.10	76
M95CE	1.25	1230	5.83	344.6	65.0	530	79		107		
		2330	11.00	679.7	80.9	840	82		111		
		0645	7.25	296.0	63.0	470	54		73		
			24.08	1320.3	71.8	1840		74		1.71	75
M100CE	2.05	1435	7.58	416.0	66.2	628	45		88		
		2305	8.50	632.1	99.7	634	60		118		
		0700	7.92	445.4	154.7	288	46		90		
			24.00	1493.5	96.4	1550		51		1.77	49
M77CE	1.50	1215	5.25	519.7	173.2	300	110		139		
		2240	10.41	608.5	155.6	391	65		82		
		0605	7.42	516.6	114.8	450	77		97		
			23.08	1644.8	144.2	1141		79		1.81	76

Appendix C5 SCr, UCr & CCr, calculated for 8 & 24h periods, for Subjects in CREATININE study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M35CE	1.54	1400	7.00	421.6	163.4	258	65		93		
		2220	8.33	611.4	178.3	343	79		113		
		0700	8.67	509.0	184.4	276	64		91		
			24.00	1542.0	175.8	877		70		1.94	62
M67CE	1.52	24.00	24.00	1208.6	64.3	1880		55		2.01	48
M57CE	1.66	24.00	24.00	1216.5	116.4	1045		51		1.78	49
M84CE	1.06	24.00	24.00	1783.0	57.5	3100		117		1.82	111
M98CE	2.25	1420	7.34	436.1	75.2	580	44		102		
		2010	5.83	387.5	161.5	240	49		114		
		0630	10.33	552.2	83.7	660	40		93		
			23.50	1375.8	93.0	1480		43		1.86	40
M79CE	1.81	1315	6.25	307.0	102.3	300	45		132		
		2230	9.25	452.9	123.1	368	45		132		
		0700	8.50	118.5	127.4	93	13		38		
			24.00	878.4	115.4	761		34		1.67	35
M38C	1.23	1410	7.17	457.4	79.4	576	86		118		
		2100	6.83	347.2	86.8	400	69		95		
		0700	10.00	485.7	138.8	350	66		90		
			24.00	1290.3	97.3	1326		73		1.60	79
M43C	1.36	24.00	24.00	1775.0	59.0	3010		91		2.10	75
M64C	1.19	1255	5.92	359.7	81.8	440	85		116		
		2300	10.08	499.9	81.3	615	69		95		
		0700	8.00	385.8	148.4	260	68		93		
			24.00	1245.4	94.7	1315		73		1.76	71
F08C	1.50	1320	7.58	302.1	39.8	760	44		142		
		2000	6.67	125.1	37.7	332	21		68		
		0545	9.75	241.3	38.2	632	27		87		
			24.00	668.5	38.8	1724		31		1.43	37
F09C	4.32	1425	6.42	191.7	84.1	228	12		120		
		2130	7.08	30.6	38.7	79	2		20		
		0730	10.00	378.5	48.4	782	15		150		
			23.50	600.8	55.2	1089		10		1.71	10
F12C	2.56	1420	8.33	389.4	120.2	324	30		125		
		2000	5.67	126.0	217.2	58	14		58		
		0600	10.00	355.9	92.7	384	23		96		
			24.00	871.3	113.7	766		24		1.85	22
F15C	1.24	1600	6.00	191.4	41.1	466	43		116		
		2040	4.67	135.5	123.2	110	39		105		
		1005	13.42	340.6	66.1	515	34		92		
			24.09	667.5	61.2	1091		37		1.67	39

Appendix C5 SCr, UCr & CCr, calculated for 8 & 24h periods, for Subjects in CREATININE study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F16C	1.19	1400	8.00	164.9	37.2	443	29		97		
		1800	4.00	97.4	17.2	566	34		113		
		0600	12.00	252.5	23.0	1100	29		97		
			24.00	514.8	24.4	2109		30		1.33	39
F17C	1.30	1515	5.75	164.3	82.2	200	37		100		
		2300	7.75	140.0	157.3	89	23		62		
		0930	10.50	384.5	108.6	354	47		127		
			24.00	688.8	107.1	643		37		2.04	31
F18C	1.81	1445	7.42	6.5	130.0	5	1		5		
		1830	3.75	254.8	172.2	148	63		332		
		0700	12.50	222.9	112.6	198	16		84		
			23.67	484.2	137.9	351		19		1.43	23
F21C	3.82	1330	6.50	120.3	101.9	118	8		62		
		1830	4.50	209.9	99.0	212	20		154		
		0700	12.50	342.9	99.7	344	12		92		
			23.50	673.1	99.9	674		13		1.47	15
F22C	1.43	1330	7.50	346.8	60.7	571	54		115		
		2047	7.28	289.2	66.0	438	46		98		
		0600	9.22	333.3	59.7	558	42		89		
			24.00	969.3	61.9	1567		47		1.68	49
F24C	3.89	1400	4.00	159.1	137.2	116	17		100		
		2200	8.00	371.6	178.7	208	20		118		
		1000	12.00	429.6	47.0	915	15		88		
			24.00	960.3	77.5	1239		17		2.04	15
F28C	1.56	24.00	24.00	314.4	22.4	1406		14		1.52	16
F25C	1.25	1415	8.25	118.3	78.9	150	19		146		
		1830	4.25	67.7	147.2	46	21		162		
		0600	11.50	53.5	100.9	53	6		46		
			24.00	239.5	96.2	249		13		1.38	17
F29C	1.22	1525	8.42	139.1	41.2	338	23		96		
		1940	4.25	85.7	99.7	86	28		117		
		0650	11.17	200.1	41.3	484	24		100		
			23.84	424.9	46.8	908		24		1.38	31
F30C	2.23	1330	7.50	250.8	97.2	258	25		100		
		2130	8.00	205.9	152.5	135	19		76		
		0600	8.50	339.4	36.5	929	30		120		
			24.00	796.1	60.2	1322		25		1.58	27
F31C	1.02	1400	8.00	207.4	68.2	304	42		100		
		1830	4.50	157.1	39.6	397	57		136		
		0600	11.50	248.5	18.3	1355	35		83		
			24.00	613.0	29.8	2056		42			

Appendix C5 SCr, UCr & CCr, calculated for 8 & 24h periods, for Subjects in CREATININE study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F34C	1.39	1300	7.00	167.3	75.4	222	29		104		
		2215	9.25	189.4	124.6	152	25		89		
		0530	7.25	201.4	51.6	390	33		118		
			23.50	558.1	73.0	764		28		2.03	24
F37C	1.62	1400	8.00	233.4	77.8	300	30		125		
		2100	7.00	252.3	95.9	263	37		154		
		0600	9.00	82.5	64.5	128	9		38		
			24.00	568.2	82.2	691		24		1.68	25
F39C	2.24	1430	8.33	268.1	75.9	353	24		104		
		1830	4.00	104.5	67.0	156	19		82		
		0600	11.50	353.0	59.3	595	23		100		
			23.83	725.6	65.7	1104		23			
F41CD	2.10	1200	5.00	92.2	31.8	290	15		107		
		2300	11.00	164.9	33.3	495	12		86		
		0700	8.00	148.0	31.5	470	15		107		
			24.00	405.1	32.3	1255		14		1.52	16
F42CD	1.82	1430	7.50	270.4	39.5	685	33		110		
		2130	7.00	253.4	105.6	240	33		110		
		0645	9.25	334.4	113.4	295	33		110		
			23.75	858.2	70.3	1220		30		1.44	35
F44CD	1.76	1300	7.25	161.2	76.8	210	21		124		
		2000	7.00	122.7	85.2	144	17		100		
		0630	10.50	159.6	67.9	235	14		82		
			24.75	443.7	75.3	589		17		1.38	21
F46CD	4.01	1330	3.50	34.5	115.0	30	4		57		
		2100	7.50	161.1	76.7	210	9		129		
		0700	10.00	175.9	59.0	298	7		100		
			21.00	371.5	69.1	538		7		1.54	8
F02GPC	1.34	24.00	24.00	915.5	58.7	1560		47		1.91	43
F04GPC	1.11	24.00	24.00	1111.3	73.4	1515		70		1.54	78
F05GPC	1.01	24.00	24.00	896.9	47.2	1900		62		1.52	70
F07GPC	1.59	24.00	24.00	882.9	42.8	2064		39		1.70	39
F09GPC	1.29	24.00	24.00	673.0	44.6	1510		36		1.54	41
M02C	1.14	23.00	23.00	634.1	41.2	1538		40		1.88	37
M03C	1.14	1310	5.17	240.3	31.0	775	68		119		
		1900	5.83	284.8	49.0	581	71		125		
		0500	10.00	294.6	51.7	570	43		75		
			21.00	819.7	42.6	1926		57		1.88	53

Appendix C5 SCr, UCr & CCr, calculated for 8 & 24h periods, for Subjects in CREATININE study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M04C	6.71	1300	4.00	124.0	139.3	89	8		80		
		2100	8.00	302.3	129.2	234	9		90		
		0500	8.00	372.2	33.2	1122	12		120		
			20.00	798.5	55.3	1445		10		1.78	10
M05C	6.71	1300	6.00	205.7	123.2	167	9		82		
		2100	8.00	289.6	108.9	266	9		82		
		0700	10.00	521.7	52.2	1000	13		118		
			24.00	1017.0	71.0	1433		11		1.78	10
M06C	1.94	1330	6.50	201.5	231.6	87	27		75		
		2030	7.00	288.5	267.1	108	35		97		
		0700	10.50	527.5	214.4	246	43		119		
			24.00	1017.5	230.7	441		36		1.88	34
M07C	0.98	1530	6.50	178.7	91.6	195	47		96		
		1930	4.00	127.6	83.9	152	54		110		
		0855	13.42	362.8	62.2	583	46		94		
			23.92	669.1	71.9	930		49		1.68	51
M10C	2.43	1210	2.42	138.4	108.1	128	39		186		
		2005	7.92	204.9	36.5	562	18		86		
		0645	10.67	285.5	47.4	602	18		86		
			21.01	628.8	48.7	1292		21		1.63	22
M13C	1.05	1400	8.00	335.8	65.6	512	67		106		
		2220	8.33	369.3	41.2	897	70		111		
		0600	7.67	246.8	65.6	376	51		81		
			24.00	951.9	53.3	1785		63		1.87	58
M14C	2.62	1500	5.50	98.8	33.2	298	11		44		
		2255	7.92	353.8	89.8	394	28		112		
		0945	10.83	473.9	55.4	856	28		112		
			24.25	926.5	59.9	1548		25		1.63	26
M19C	2.05	24.00	24.00	415.6	51.7	804		14		1.54	16
M20C	4.86	1500	9.00	263.8	77.6	340	10		83		
		2200	7.00	218.8	124.3	176	11		92		
		0600	8.00	333.4	45.7	730	14		117		
			24.00	816.0	65.5	1246		12		1.78	11
M23C	1.77	1430	7.50	233.3	57.5	406	29		121		
		2045	6.25	133.8	77.8	172	20		83		
		0740	10.92	251.1	163.1	154	22		92		
			24.67	618.2	84.5	732		24		1.66	25
M26C	1.66	1440	8.42	241.3	86.2	280	29		67		
		1645	2.08	244.2	290.7	84	118		274		
		0640	13.92	548.0	50.9	1076	40		93		
			24.42	1033.5	71.8	1440		43		1.72	43

Appendix C5 SCr, UCr & CCr, calculated for 8 & 24h periods, for Subjects in CREATININE study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M27C	1.51	1135	5.58	615.7	150.9	408	122		290		
		1530	3.92	262.4	145.8	180	74		176		
		0600	14.50	41.9	130.9	32	3		7		
			24.00	920.0	148.4	620		42		1.80	41
M43CD	1.52	1425	2.42	280.3	59.6	470	127		259		
		2010	5.75	162.7	29.7	548	31		63		
		1000	13.83	549.2	140.5	391	44		90		
			22.00	992.2	70.4	1409		49		2.03	38
M01GPC	1.47	24.00	24.00	1385.6	99.7	1390		65		1.80	63
M03GPC	1.35	24.00	24.00	1305.4	77.5	1685		67		2.07	56
M06GPC	1.30	24.00	24.00	1559.4	131.6	1185		83		1.80	80
M08GPC	1.50	24.00	24.00	1492.7	130.9	1140		69		1.91	63
M10GPC	1.97	24.00	24.00	1257.0	84.1	1495		44		1.92	40
M11GPC	1.60	24.00	24.00	1110.0	61.0	1820		48		1.78	47

Appendix C6 Females (F) v Males (M) in 24h & 8h CCr Study - Mann-Whitney Test

	F min	F max	F median	F mean	F s.d.	M min	M max	M median	M mean	M s.d.	
age (years)	60	100	78.0	77.1	8.4	60	97	78.0	76.3	8.2	
mobility score	1	5	1.0	2.1	1.3	1	5	1.0	2.2	1.4	.
height (cm)	137	178	157.0	157.4	7.9	147	187	172.0	171.5	7.3	****
weight (kg)	36	94	60.5	60.8	12.8	40	96	71.0	71.0	12.1	****
LBM (kg)	30	57	42.1	42.2	5.7	34	60	50.0	50.0	5.6	****
SA (m2)	1.23	2.15	1.63	1.64	0.22	1.36	2.20	1.82	1.83	0.18	****
urine volume (ml)	249	3217	1333	1375	584	360	3742	1440	1517	641	
UCr (mg in 24h)	240	2441	834	823	313	302	2624	1223	1192	433	****
[UCr] (mg/100ml)	22.4	420.8	60.8	67.9	39.7	26.9	240.6	77.5	87.3	39.7	****
[SCr] (mg/100ml)	0.34	4.32	1.24	1.39	0.69	0.55	6.71	1.45	1.67	1.01	**
24h CCr (ml/min)	7	141	47.0	48.7	24.1	10	193	59.0	60.5	30.0	***
24h CCr/SA (ml/min/1.73m2)	8	167	50.0	51.8	26.3	10	164	53.0	56.4	26.1	
CCram (ml/min)	1	160	49.0	50.5	30.4	6	195	63.5	67.6	41.1	**
CCrpm (ml/min)	2	156	49.0	51.9	31.6	9	209	61.5	63.2	36.9	*
CCrn (ml/min)	6	153	43.0	46.1	26.2	3	186	51.5	53.9	30.3	

* p<0.05 ** p<0.005 *** p<0.001 **** p<0.0001

Appendix C7 Summary of Results From All Females in CCr Prediction Study

	n	min	max	median	mean	s.d.	95% C.I.	r	m	c
age (years)	146	60	100	78.0	77.1	8.4	76 - 78			
mobility score	146	1	5	1.0	2.1	1.3	1.9 - 2.3			
height (cm)	146	137	178	157.0	157.4	7.9	156 - 159			
weight (kg)	146	36	94	60.5	60.8	12.8	59 - 63			
SA (m2)	146	1.23	2.15	1.63	1.64	0.22	1.60 - 1.67			
urine vol (ml)	146	249	3217	1333	1375	584	1279 - 1470			
time interval (h)	146	21.00	25.42	24.00	23.88	0.52	23.79-23.96			
UCr (mg/24h)	146	240	2441	834	823	313	772 - 874			
[UCr] (mg/100ml)	146	22	421	60.8	67.9	39.7	61.4 - 74.4			
[SCr] (mg/100ml)	146	0.34	4.32	1.24	1.39	0.69	1.27 - 1.50			
24h CCr (ml/min)	116	7	141	46.5	48.5	26.1	44 - 53			
CCram (ml/min)	116	1	160	49.0	50.5	30.4	45 - 56	0.901	1.05	-0.45
dCCram	116	-57	36	-1.0	-2.0	13.3	4.5 - 0.5			
%CCram	116	5	263	102.5	102.6	32.0	97 - 109			
CCrpm (ml/min)	116	2	156	49.0	51.9	31.6	46 - 58	0.877	1.06	0.37
dCCrpm	116	-80	39	-2.0	-3.3	15.3	-6.1 - -0.5			
%CCrpm	116	20	332	103.0	107.4	39.8	100 - 115			
CCrn (ml/min)	116	6	153	43.0	46.1	26.2	41 - 51	0.922	0.93	1.21
dCCrn	116	-41	42	2.0	2.4	10.3	0.5 - 4.3			
%CCrn	116	27	241	97.0	96.4	25.1	92 - 101			
CCr (ml/min)	146	7	141	47.0	48.7	24.1	45 - 53			
CCr/SA (ml/min/1.73m2)	146	8	167	50.0	51.8	26.3	48 - 56			
CCr/70kg (ml/min/70kg)	146	9	207	52.5	57.1	30.9	50 - 62			
E1 (ml/min)	146	6	133	44.9	45.8	20.5	42 - 49	0.826	0.7	11.6
dE1		-41	44	2.1	2.9	13.6				
E1F (ml/min)	146	5	113	38.1	38.9	17.4	36 - 42	0.826	0.6	9.9
dE1F		-26	48	7.9	9.8	13.8				
E2 (ml/min/1.73m2)	146	11	282	68.7	75.0	39.9	68 - 82	0.779	1.18	13.7
dE2		-124	35	-17.8	-23.2	25.5				
E2F (ml/min/1.73m2)	146	11	228	57.5	62.6	31.9	57 - 68	0.779	0.95	13.6
dE2F		-70	38	-6.4	-10.8	20.1				

Appendix C7 Summary of Results From All Females in CCr Prediction Study (contd)

	n	min	max	median	mean	s.d.	95% C.I.	r	m	c
E3 (ml/min/1.73m2)	146	10	159	43.2	45.4	20.9	42 - 49	0.844	0.67	10.6
dE3		-28	43	7.1	6.4	14.2				
E3F (ml/min/1.73m2)	146	9	143	38.9	40.9	18.8	38 - 44	0.844	0.61	9.6
dE3F		-21	51	11.5	10.9	14.5				
E4 (ml/min)	146	7	149	49.8	51.2	22.8	47 - 55	0.826	0.78	13.1
dE4		-53	42	-2.4	-2.5	13.9				
E4F (ml/min)	146	6	126	42.4	43.5	19.4	40 - 47	0.826	0.67	11.1
dE4F		-36	45	4.3	5.2	13.6				
E5 (ml/min/1.73m2)	146	20	276	74.3	80.2	37.6	74 - 86	0.779	1.11	22.5
dE5		-118	28	-23.4	-28.4	23.8				
E6 (ml/min)	146	16	329	70.6	78.9	44.7	72 - 86	0.696	1.29	16.1
dE6		-202	36	-22.5	-30.2	32.9				
E6F (ml/min)	146	13	253	54.9	61.2	34.4	56 - 67	0.774	1.01	8.8
dE6F		-126	42	-6.9	-12.6	24.7				
E7 (ml/min/1.73m2)	146	86	118	103.2	103.9	6.7	103 - 105	0.293	0.07	100
dE7		-101	61	-56.2	-52.1	25.2				
E8 (ml/min/1.73m2)	146	69	95	83.1	83.6	5.3	83 - 85	0.293	0.06	80.6
dE8		-80	82	-35.3	-31.8	25.2				
E9 (ml/min)	146	7	144	48.2	49.5	22.1	46 - 53	0.825	0.76	12.7
dE9		-49	43	-1.1	-0.8	13.8				
E9F (ml/min)	146	6	115	38.5	39.6	17.7	37 - 42	0.825	0.60	10.2
dE9F		-28.2	46.9	7.5	9.1	13.8				
E10 (ml/min/70kg)	146	10	203	53.0	56.0	27.1	52 - 60	0.858	0.75	12.9
dE10		-40	48	-0.1	1.2	15.9				
E10F (ml/min/70kg)	146	9	172	45.1	47.6	23.0	44 - 51	0.858	0.64	11.0
dE10F		-27	56	8.5	9.6	16.2				
E11 (ml/min)	146	13	297	60.8	68.7	39.5	62 - 75	0.726	1.19	10.8
dE11		-167	41	-15.5	-20.0	27.6				
E11F (ml/min)	146	12	178	42.7	46.9	23.5	43 - 51	0.716	0.70	12.9
dE11F		-54	49	3.8	1.8	18.0				
E12F (ml/min)	143	8	86	39.0	40.6	16.1	38 - 43	0.784	0.57	13.2
dE12F		-31	46	7.0	7.1	13.7				

Appendix C8 Correlation Coefficient for All Females in CCr Prediction Study (n=146)

	age	M.Sc	weight	SA	U vol.	UCr	[SCr]	CCr
mobility score	0.513 ***							
weight (kg)	-0.330 ***	-0.192						
SA (m2)	-0.180	-0.079	0.804 ***					
urine volume (ml)	-0.438 ***	-0.441 ***	0.357 ***	0.247 **				
UCr (mg)	-0.463 ***	-0.421 ***	0.468 ***	0.339 ***	0.464 ***			
[SCr] (mg/100ml)	0.116	0.306 ***	0.033	0.140	-0.138	0.001		
CCr (ml/min)	-0.358 ***	-0.438 ***	0.204	0.094	0.274 ***	0.561 ***	-0.619 ***	
CCr/SA (ml/min/1.73m2)	-0.293 ***	-0.394 ***	0.002	-0.081	0.194	0.446 ***	-0.636 ***	0.973 ***

** p<0.01 *** p<0.001

Appendix C9 Summary of Results From All Males in CCr Prediction Study

	n	min	max	median	mean	s.d.	95% C.I.	r	m	c
age (years)	99	60	97	78.0	76.3	8.2	74 - 78			
mobility score	99	1	5	1.0	2.2	1.4	1.9 - 2.5			
height (cm)	99	147	187	172.0	171.5	7.3	170 - 173			
weight (kg)	99	40	96	71.0	71.0	12.1	69 - 73			
SA (m2)	99	1.36	2.20	1.82	1.83	0.18	1.79 - 1.87			
urine vol (ml)	99	360	3742	1440	1517	641	1389 - 1645			
time interval (h)	99	20.00	27.33	24.00	23.89	0.90	23.71 - 24.07			
UCr (mg/24h)	99	302	2624	1223	1192	433	1106 - 1278			
[UCr] (mg/100ml)	99	27	241	77.5	87.3	39.7	79.4 - 95.2			
[SCr] (mg/100ml)	99	0.55	6.71	1.45	1.67	1.01	1.47 - 1.87			
24h CCr (ml/min)	78	10	193	57.5	59.6	31.4	53 - 67			
CCram (ml/min)	78	6	195	63.5	67.6	41.1	58 - 77	0.846	1.11	1.58
dCCram	78	-87	17	0.0	-8.0	22.1	-13.0--3.0			
%CCram	78	30	350	100.0	112.0	48.6	101 - 123			
CCrpm (ml/min)	78	9	209	61.5	63.2	36.9	55 - 72	0.925	1.09	-1.52
dCCrpm	78	-75	29	-2.0	-3.6	14.3	-6.8 - -0.4			
%CCrpm	78	37	274	106.5	105.4	29.8	99 - 112			
CCrn (ml/min)	78	3	186	51.5	53.9	30.3	47 - 61	0.905	0.87	1.78
dCCrn	78	-43	50	3.0	5.7	13.5	2.7 - 8.8			
%CCrn	78	7	149	93.5	92.4	22.0	87 - 97			
CCr (ml/min)	99	10	193	59.0	60.5	30.0	55 - 67			
CCr/SA (ml/min/1.73m2)	99	10	164	53.0	56.4	26.1	51 - 62			
CCr/70kg (ml/min/70kg)	99	10	169	54.7	59.2	27.0	54 - 65			
E1 (ml/min)	99	8	145	44.3	46.8	22.2	42 - 51	0.862	0.64	8.2
dE1		-27	51	13.3	13.8	15.7				
E2 (ml/min/1.73m2)	99	3	170	57.0	61.7	30.6	56 - 68	0.726	0.85	13.7
dE2		-88	44	0.3	-5.3	21.4				
E3 (ml/min/1.73m2)	99	7	108	37.6	39.3	17.6	36 - 43	0.781	0.53	9.7
dE3		-31.6	55.6	16.8	17.1	16.6				
E4 (ml/min)	99	9	162	49.6	52.2	24.7	47 - 57	0.861	0.71	9.3
dE4		-36	48	6.7	8.4	15.3				

Appendix C9 Summary of Results From All Males in CCr Prediction Study (contd)

	n	min	max	median	mean	s.d.	95% C.I.	r	m	c
E5 (ml/min/1.73m2)	99	12	170	63.2	67.7	28.9	62 - 73	0.726	0.80	22.4
dE5		-90	37	-6.1	-11.3	20.5				
E6 (ml/min)	99	9	186	58.6	64.6	32.1	58 - 71	0.670	0.72	21.3
dE6		-103.6		1.0	-4.1	25.3				
E7 (ml/min/1.73m2)	99	88	118	103.2	104.5	6.5	104 - 106	0.420	0.11	98.6
dE7		-92	52	-48.8	-48.1	24.1				
E8 (ml/min/1.73m2)	99	71	95	83.1	84.1	5.2	83 - 85	0.420	0.08	79.4
dE8		-72	75	-28.8	-27.7	24.4				
E9 (ml/min)	99	8	156	48.0	50.4	23.9	46 - 55	0.861	0.69	9.0
dE9		-33	49	8.8	10.1	15.4				
E10 (ml/min/70kg)	99	7	137	45.7	48.0	22.7	43 - 53	0.804	0.68	8.0
dE10		-42.5	58	10.4	11.2	16.1				
E11 (ml/min)	99	8	172	50.7	56.5	29.0	51 - 62	0.694	0.67	15.9
dE11		-82	53	7.6	4.0	23.1				
E12 (ml/min)	97	12	148	47.0	48.5	22.1	44 - 53	0.844	0.63	9.6
dE12		-32	52	13.0	13.1	16.0				

Appendix C10 Correlation Coefficient for All Males in CCr Prediction Study (n=99)

	age	M.Sc	weight	SA	U vol.	UCr	[SCr]	CCr
mobility score	0.423 ***							
weight (kg)	-0.253	-0.376 ***						
SA (m2)	-0.269 **	-0.381 ***	0.947 ***					
urine volume (ml)	-0.329 ***	-0.288 **	0.362 ***	0.385 ***				
UCr (mg)	-0.442 ***	-0.550 ***	0.566 ***	0.585 ***	0.431 ***			
[SCr] (mg/100ml)	0.206	0.292 **	-0.016	-0.049	-0.097	-0.094		
CCr (ml/min)	-0.421 ***	-0.512 ***	0.424 ***	0.476 ***	0.438 ***	0.610 ***	-0.593 ***	
CCr/SA (ml/min/1.73m2)	-0.420 ***	-0.499 ***	-0.274	0.321 **	0.406 ***	0.555 ***	-0.634 ***	0.980 ***

** p<0.01 *** p<0.001

Appendix C11 Influence of Diuretic Use on CCr Prediction in Females - Mann-Whitney Test

	no diuretics taken (no D; n=86)					diuretics taken (D; n=60)					
	no D mean	no D s.d.	no D r	no D m	no D c	D mean	D s.d.	D r	D m	D c	p
age (years)	75.6	8.2				79.3	8.2				**
mobility score	1.9	1.2				2.4	1.3				*
weight (kg)	60.4	11.0				61.3	15.1				
SA (m2)	1.62	0.17				1.66	0.27				
urine volume (ml)	1423	592				1306	570				
UCr (mg in 24h)	152	326				782	291				
[UCr] (mg/100ml)	70.1	47.9				64.8	23.6				
[SCr] (mg/100ml)	1.31	0.60				1.49	0.79				
CCr (ml/min)	52.1	26.0				43.9	20.5				
CCr/SA (ml/min/1.73m2)	29.0	29.1				21.9	20.6				
CCram (ml/min)	55.2	34.2	0.942	1.11	-3.8	44.9	24.4	0.795	0.92	5.4	
dCCram	-2.1	11.9				-1.9	14.9				
CCrpm (ml/min)	58.6	33.3	0.865	0.99	5.8	43.8	27.5	0.890	1.16	-6.2	**
dCCrpm	-5.5	16.7				-0.8	13.0				
CCrn (ml/min)	49.9	29.0	0.957	0.96	-0.9	41.7	21.9	0.841	0.88	4.0	
dCCrn	3.3	8.5				1.3	12.2				*
E1 (ml/min)	47.7	20.0	0.844	0.65	13.8	43.0	21.0	0.811	0.83	6.7	
dE1	4.4	14.1				0.8	12.7				
E4 (ml/min)	53.2	22.3	0.844	0.73	15.4	48.2	23.5	0.810	0.93	7.6	
dE4	-1.2	13.9				-4.4	13.8				
E9 (ml/min)	51.4	21.6	0.844	0.70	15.0	46.6	22.7	0.810	0.90	7.4	
dE9	0.62	13.9				-2.8	13.5				
E10 (ml/min/70kg)	58.9	28.8	0.885	0.74	13.5	51.7	24.1	0.792	0.82	10.3	
dE10	2.7	16.2				-1.0	15.3				
E11F (ml/min)	48.9	25.1	0.730	0.71	12.2	43.9	20.9	0.674	0.69	13.8	
dE11F	3.1	18.8				0.0	16.7				

* $p<0.05$ ** $p<0.02$

Appendix C12 Influence of Diuretic Use on CCr Prediction in Males - Mann-Whitney Test

	no diuretics taken (no D; n=63)					diuretics taken (D; n=36)					
	no D mean	no D s.d.	no D r	no D m	no D c	D mean	D s.d.	D r	D m	D c	p
age (years)	75.2	8.6				78.3	7.2				
mobility score	1.9	1.4				2.8	1.2				**
weight (kg)	70.1	12.1				72.5	12.0				
SA (m2)	1.82	0.18				1.85	0.17				
urine vol (ml)	1457	641				1622	638				
UCr (mg/24h)	1213	441				1155	423				
[UCr] (mg/100ml)	93.7	44.5				76.1	26.5				
[SCr] (mg/100ml)	1.48	0.57				1.99	1.46				
CCr (ml/min)	63.4	25.6				55.5	36.3				
CCr/SA (ml/min/1.73m2)	59.6	22.3				50.8	31.4				*
CCram (ml/min)	68.4	34.3	0.841	1.06	2	66.6	48.7	0.853	1.15	2	
dCCram	-6.4	18.6				-10.0	26.0				
CCrpm (ml/min)	67.1	30.1	0.868	0.96	7	58.5	43.8	0.962	1.16	-7	
dCCrpm	-5.1	15.0				-1.8	3.4				
CCrn (ml/min)	56.2	27.3	0.934	0.94	-2	51.0	33.9	0.883	0.83	4	
dCCrn	5.8	9.8				5.7	17.1				
E1 (ml/min)	48.5	19.2	0.781	0.59	11	43.8	26.7	0.932	0.68	6	
dE1	15.0	16.0				11.7	15.0				
E4 (ml/min)	54.0	21.4	0.780	0.65	13	48.9	29.7	0.932	0.76	7	
dE4	9.4	16.1				6.6	13.9				
E9 (ml/min)	52.3	20.7	0.780	0.63	12	47.3	28.7	0.932	0.74	6	
dE9	11.2	16.1				8.2	14.2				
E10 (ml/min/70kg)	50.6	20.4	0.694	0.62	12	43.6	26.0	0.903	0.72	5	
dE10	12.1	17.0				9.6	14.4				
E11 (ml/min)	59.3	26.9	0.515	0.54	25	51.7	32.2	0.876	0.78	9	
dE11	4.1	25.9				3.8	17.5				

* $p<0.05$ ** $p<0.002$

Appendix C13 Influence of 24h CCr on prediction of CCr in Females - Mann-Whitney Test

	CCr/SA > 50ml/min/1.73m2 (n=75)					CCr/SA < 50ml/min/1.73m2 (n=71)					
	> 50 mean	> 50 s.d.	> 50 r	> 50 m	> 50 c	< 50 mean	< 50 s.d.	< 50 r	< 50 m	< 50 c	p
age (years)	74.3	8.1				80.1	7.6				**
mobility score	1.5	0.9				2.8	1.3				**
weight (kg)	62.0	11.1				59.4	14.4				
SA (m2)	1.64	0.16				1.63	0.27				
urine volume (ml)	1522	551				1220	582				*
UCr (mg in 24h)	987	280				649	247				**
[SCr] (mg/100ml)	1.09	0.35				1.70	0.81				**
24h CCr (ml/min)	66.4	18.6				30.0	12.2				**
24h CCr/SA (ml/min/1.73m2)	70.6	22.3				32.0	11.5				**
dCCram	-2.2	15.2	0.825	1.07	-2.9	-1.9	11.4	0.807	1.21	-4.3	
%CCram	104.8	23.3				101.0	37.4				
dCCrpm	-4.9	17.2	0.783	1.05	1.6	-1.7	13.2	0.679	0.98	2.2	
%CCrpm	107.3	26.7				107.5	47.6				
dCCrn	4.3	11.7	0.861	0.96	-1.6	0.5	8.6	0.806	0.93	1.4	
%CCrn	93.8	16.0				98.3	30.2				
dE1	10.1	12.5	0.773	0.77	5.4	-4.7	10.2	0.776	1.03	3.7	**
%E1	122.2	35.8				97.1	32.6				**
dE4	3.6	13.3	0.775	0.86	5.9	-8.8	11.6	0.775	1.15	4.3	**
%E4	109.5	31.9				86.6	28.9				**
dE9	5.6	13.0	0.775	0.83	5.7	-7.5	11.2	0.775	1.11	4.1	**
%E9	113.2	33.0				89.5	29.9				**
dE10	8.5	14.9	0.866	0.84	3.9	-6.6	13.0	0.668	0.92	9.6	**
%E10	116.0	36.5				94.8	33.6				**
dE11F	9.8	17.2	0.746	1.03	-12.1	-6.7	14.7	0.455	0.57	19.4	**
%E11F	130.5	40.2				89.2	33.8				**

* p<0.001 ** p<0.0001

Appendix C14 Influence of 24h CCr on prediction of CCr in Males - Mann-Whitney Test

	CCr/SA > 50ml/min/1.73m2 (n=58)					CCr/SA < 50ml/min/1.73m2 (n=41)					
	> 50 mean	> 50 s.d.	> 50 r	> 50 m	> 50 c	< 50 mean	< 50 s.d.	< 50 r	< 50 m	< 50 c	p
age (years)	74.0	7.0				79.6	8.7				***
mobility score	1.7	1.1				2.9	1.4				****
weight (kg)	73.6	11.6				67.2	11.9				*
SA (m2)	1.87	0.17				1.77	0.18				*
urine volume (ml)	1675	715				1294	437				*
UCr (mg in 24h)	1361	411				953	346				****
[UCr] (mg/100ml)	92.7	41.6				79.5	35.9				
[SCr] (mg/100ml)	1.24	0.35				2.28	1.30				****
24h CCr (ml/min)	79.3	23.1				34.0	14.6				****
24h CCr/SA (ml/min/1.73m2)	73.3	18.9				32.5	12.9				****
dCCram	-9.8	21.5	0.791	1.10	2	-5.8	23.8	0.592	1.25	-2	
%CCram	111.3	26.1				113.9	69.8				
dCCrpm	-4.4	12.3	0.925	1.16	-8	-2.6	17.0	0.672	1.11	-1	
%CCrpm	104.6	17.3				106.5	42.2				
dCCrn	8.7	15.3	0.830	0.92	-2	1.7	9.4	0.793	0.86	3	**
%CCrn	88.4	18.5				97.5	25.6				**
dE1	20.7	15.6	0.744	0.64	8	30.0	12.6	0.780	0.68	7	****
%E1	142.5	39.6				116.3	30.7				****
dE4	14.0	16.2	0.744	0.71	9	0.5	9.6	0.777	0.75	8	****
%E4	127.7	35.4				104.0	27.5				****
dE9	16.1	16.0	0.744	0.68	9	1.6	0.5	0.778	0.73	8	****
%E9	132.1	36.6				107.5	106.9				****
dE10	16.9	16.8	0.667	0.67	8	3.2	10.9	0.696	0.76	5	****
%E10	136.3	39.9				117.7	33.2				*
dE11	9.5	24.7	0.533	0.63	20	-3.7	18.2	0.433	0.58	18	****
%E11	123.2	39.2				99.4	36.0				**

* p<0.05 ** p<0.006 ***p<0.001 **** p<0.0003

Appendix C15 All Fit v Frail Females in CCr Prediction Study - Mann-Whitney Test

	all fit females (n=91)					all frail females (n=55)					
	all fit mean	all fit s.d.	all fit r	all fit m	all fit c	frail mean	frail s.d.	frail r	frail m	frail c	p
age (years)	74.0	7.5				82.4	7.0				****
mobility score	1.2	0.4				3.6	0.6				****
weight (kg)	62.8	11.2				57.5	14.6				***
SA (m2)	1.65	0.16				1.61	0.29				*
urine volume (ml)	1578	517				1039	536				****
UCr (mg in 24h)	934	239				639	335				****
[SCr] (mg/100ml)	1.24	0.42				1.63	0.94				*
24h CCr (ml/min)	56.6	19.5				35.5	25.5				****
24h CCr/SA (ml/min/1.73m2)	59.6	20.7				39.0	29.5				****
dCCram	-0.3	13.3	0.867	1.05	-2.5	-4.2	13.1	0.921	1.14	-1.1	
%CCram	99.4	24.7				106.5	39.1				
dCCrpm	-3.8	11.9	0.882	1.01	3.0	-2.8	18.7	0.841	1.11	-1.2	
%CCrpm	107.9	23.7				106.9	53.6				
dCCrn	2.5	11.1	0.877	0.91	2.8	2.3	9.4	0.934	0.91	0.9	
%CCrn	96.6	25.8				96.1	24.4				
dE1	6.4	12.5	0.768	0.63	14.4	-2.8	13.5	0.856	0.83	9.0	****
%E1	114.8	27.4				98.4	45.9				****
dE4	0.6	12.7	0.771	0.71	16.1	-7.6	14.4	0.856	0.93	10.1	***
%E4	102.9	24.3				87.5	40.9				****
dE9	2.5	12.6	0.770	0.68	15.5	-6.2	14.1	0.856	0.90	9.8	****
%E9	106.4	25.1				90.5	42.2				****
dE10	5.1	14.3	0.809	0.63	18.8	-5.3	16.4	0.860	0.90	9.8	****
%E10	109.5	26.2				96.3	48.0				****
dE11F	8.8	13.9	0.712	0.59	14.7	-9.6	18.3	0.855	1.05	7.7	****
%E11F	121.0	30.7				87.1	49.7				****

* p<0.05 ** p<0.005 *** p<0.0005 **** p<0.0001

Appendix C16 Very Fit v Fit Females - CCr Prediction Study - Mann-Whitney Test

	very fit females (n=17)					fit females (n=17)					
	v.fit mean	v.fit s.d.	v.fit r	v.fit m	v.fit c	fit mean	fit s.d.	fit r	fit m	fit c	p
age (years)	69.4	4.5				69.1	4.7				
mobility score	1.0	0.0				1.1	0.2				
weight (kg)	61.5	9.2				65.9	9.8				
SA (m2)	1.63	0.13				1.70	0.13				
urine volume (ml)	1613	500				1605	496				
UCr (mg in 24h)	1000	139				981	217				
[SCr] (mg/100ml)	1.27	0.49				1.17	0.22				
24h CCr (ml/min)	59.4	15.5				61.2	21.1				
24h CCr/SA (ml/min/1.73m2)	63.6	18.9				62.5	21.9				
dCCram	-3.2	3.3	0.978	0.97	5.4	6.1	12.0	0.928	1.1	-15.0	*
%CCram	105.3	5.3				87.4	22.2				*
dCCrpm	-0.1	4.4	0.967	1.0	-2.7	-8.6	10.8	0.919	0.99	9.2	
%CCrpm	100.1	6.6				115.4	19.2				
dCCrn	1.8	3.5	0.965	1.0	-2.8	2.0	4.6	0.986	0.90	4.8	
%CCrn	97.0	5.6				98.1	5.8				
dE1	7.5	14.1	0.565	0.53	20.4	3.9	12.9	0.800	0.55	24.0	
%E1	118.6	26.7				105.6	20.5				
dE4	1.8	14.7	0.570	0.59	22.3	-2.5	12.7	0.802	0.61	26.5	
%E4	106.8	23.9				95.1	18.4				
dE9	3.7	14.5	0.570	0.57	21.6	-0.4	12.7	0.802	0.59	25.7	
%E9	110.5	24.7				98.3	19.0				
dE10	7.4	17.0	0.713	0.50	27.4	1.3	12.7	0.889	0.59	25.8	
%E10	113.5	26.1				100.5	19.1				
dE11F	11.5	8.3	0.847	0.73	4.7	11.7	13.6	0.791	0.47	20.8	
%E11F	126.7	20.2				123.0	26.9				

* $p<0.03$

Appendix C17 Fit v Frail Females - CCr Prediction Study - Mann-Whitney Test

	fit females (n=38)					frail females (n=38)					
	fit mean	fit s.d.	fit r	fit m	fit c	frail mean	frail s.d.	frail r	frail m	frail c	p
age (years)	79.8	5.9				79.9	6.1				
mobility score	1.3	0.5				3.6	0.6				****
weight (kg)	62.0	13.0				59.0	14.8				
SA (m2)	1.64	0.19				1.61	0.24				
urine volume (ml)	1422	488				1082	604				**
UCr (mg in 24h)	851	256				661	371				***
[SCr] (mg/100ml)	1.28	0.50				1.63	1.01				
24h CCr (ml/min)	52.0	22.3				37.7	28.5				**
24h CCr/SA (ml/min/1.73m2)	54.8	22.9				41.3	34.0				***
dCCram	0.4	14.7	0.863	1.06	-3.9	-4.5	14.7	0.915	1.12	-0.4	
%CCram	97.4	25.3				108.2	43.0				
dCCrpm	-3.8	14.1	0.869	1.05	0.9	-4.5	21.9	0.827	1.10	0.4	
%CCrpm	108.0	28.2				110.4	61.4				
dCCrn	1.9	13.2	0.841	0.84	7.0	3.6	10.5	0.933	0.92	-0.6	
%CCrn	98.9	30.8				92.8	25.6				
dE1	6.5	12.8	0.818	0.67	10.8	-3.7	15.2	0.849	0.79	11.6	*
%E1	115.8	28.4				94.5	47.2				***
dE4	0.9	13.0	0.819	0.75	12.2	-8.7	16.1	0.849	0.89	13.0	*
%E4	103.2	25.1				84.3	42.1				***
dE9	2.6	12.9	0.819	0.72	11.8	-7.2	15.7	0.849	0.86	12.6	*
%E9	106.7	26.0				87.2	43.6				***
dE10	5.4	14.4	0.829	0.64	16.4	-6.4	17.5	0.872	0.88	12.1	**
%E10	110.4	27.0				92.2	48.4				**
dE11F	5.5	15.3	0.731	0.59	16.0	-10.3	19.7	0.841	1.07	7.5	****
%E11F	114.0	33.6				88.5	55.7				***

* p<0.002 ** p<0.001 *** p<0.0005 **** p<0.0001

Appendix C18 Very Fit v Frail Females - CCr Prediction Study - Mann-Whitney Test

	very fit females (n=8)					frail females (n=8)					
	v.fit mean	v.fit s.d.	v.fit r	v.fit m	v.fit c	frail mean	frail s.d.	frail r	frail m	frail c	p
age (years)	72.5	4.2				72.4	4.6				
mobility score	1.0	0.0				3.6	0.5				
weight (kg)	62.4	10.1				60.1	16.9				
SA (m2)	1.64	0.13				1.62	0.32				
urine volume (ml)	1721	525				1043	498				
UCr (mg in 24h)	1035	113				629	210				***
[SCr] (mg/100ml)	1.31	0.69				1.56	1.37				
24h CCr (ml/min)	61.8	16.2				43.6	41.0				*
24h CCr/SA (ml/min/1.73m2)	66.0	20.6				48.4	49.8				*
dCCram	-1.5	4.0	0.893	0.96	4.6	-18.0	27.8	0.919	1.07	14.1	
%CCram	102.0	5.7				132.5	90.1				
dCCrpm	-1.3	5.2	0.855	1.02	-0.5	7.0	7.4	0.992	0.99	-6.3	
%CCrpm	101.8	7.1				88.2	32.1				
dCCrn	0.8	4.0	0.883	0.84	11.8	0.5	15.2	0.980	1.12	-6.9	
%CCrn	99.3	5.3				93.0	38.9				
dE1	11.7	10.0	0.787	0.62	12.0	-7.3	11.5	0.965	0.83	14.5	**
%E1	125.8	20.5				79.4	25.2				**
dE4	6.0	10.1	0.790	0.69	12.9	-13.0	10.7	0.965	0.94	15.9	**
%E4	113.0	13.8				71.3	22.6				**
dE9	7.9	10.0	0.790	0.67	12.5	-11.2	10.8	0.965	0.90	15.4	**
%E9	116.9	19.0				73.8	23.4				**
dE10	11.8	13.6	0.903	0.61	16.0	-11.2	12.5	0.978	0.94	14.4	***
%E10	120.6	20.9				76.3	22.6				**
dE11F	13.9	4.8	0.955	0.93	-9.4	-11.7	16.8	0.961	1.21	2.4	****
%E11F	133.8	18.6				80.8	26.5				***

* p<0.05 ** p<0.01 *** p<0.005 **** p<0.002

Appendix C19 All Fit v Frail Males in CCr Prediction Study - Mann-Whitney Test

	all fit males (n=57)					all frail males (n=42)					
	all fit mean	all fit s.d.	all fit r	all fit m	all fit c	frail mean	frail s.d.	frail r	frail m	frail c	p
age (years)	73.6	7.5				80.1	7.6				****
mobility score	1.1	0.4				3.7	0.7				****
weight (kg)	74.0	10.4				66.9	13.1				***
SA (m2)	1.88	0.16				1.77	0.18				***
urine volume (ml)	1651	610				1336	645				**
UCr (mg in 24h)	1371	286				950	483				****
[SCr] (mg/100ml)	1.42	0.40				2.00	1.43				
24h CCr (ml/min)	72.5	26.2				44.3	27.3				****
24h CCr/SA (ml/min/1.73m2)	66.7	22.0				42.5	25.0				****
dCCram	-4.3	15.3	0.898	1.06	-0.4	-11.7	27.1	0.828	1.35	-4.4	
%CCram	104.3	21.3				119.7	64.9				
dCCrpm	-5.4	9.6	0.957	1.07	0.3	-1.8	17.7	0.861	1.08	-2.0	*
%CCrpm	108.0	13.9				102.9	39.9				
dCCrn	7.0	10.6	0.933	0.93	-1.5	4.5	15.9	0.828	0.80	4.7	
%CCrn	89.8	16.1				95.0	26.6				
dE1	20.5	11.6	0.912	0.68	2.5	4.7	16.0	0.811	0.70	8.4	****
%E1	141.3	22.8				118.5	49.8				****
dE4	14.5	10.9	0.914	0.76	2.9	0.0	16.6	0.811	0.79	9.5	****
%E4	126.8	20.0				105.9	44.7				****
dE9	16.5	11.0	0.914	0.73	2.8	1.5	16.3	0.811	0.76	1.2	****
%E9	131.1	20.7				109.5	46.2				****
dE10	17.2	10.9	0.884	0.73	1.7	3.0	18.4	0.760	0.75	8.3	****
%E10	136.0	22.1				118.5	51.4				****
dE11	13.1	16.9	0.785	0.75	5.0	-8.4	24.8	0.686	0.84	15.3	****
%dE11	126.5	26.3				95.5	47.2				****

* p<0.05 ** p<0.01 ***p<0.005 **** p<0.0002

Appendix C20 Very Fit v Fit Males - CCr Prediction Study - Mann-Whitney Test

	very fit males (n=12)					fit males (n=12)					
	v.fit mean	v.fit s.d.	v.fit r	v.fit m	v.fit c	fit mean	fit s.d.	fit r	fit m	fit c	p
age (years)	68.5	3.2				68.7	3.3				
mobility score	1.0	0.0				1.3	0.8				
weight (kg)	76.4	8.5				69.8	14.2				
SA (m2)	1.91	0.13				1.81	0.24				
urine volume (ml)	1765	743				1437	526				
UCr (mg in 24h)	1573	230				1225	381				**
[SCr] (mg/100ml)	1.47	0.32				1.38	0.37				
24h CCr (ml/min)	78.3	23.3				65.7	20.1				
24h CCr/SA (ml/min/1.73m2)	70.8	20.0				62.5	15.8				
dCCram	-4.0	15.2	0.896	1.20	-10.9	-4.9	16.5	0.937	1.39	-19.9	
%CCram	102.9	20.7				104.9	23.2				
dCCrpm	-5.3	9.9	0.913	0.83	18.5	-7.6	9.0	0.958	1.11	0.3	
%CCrpm	110.0	17.6				113.0	12.9				
dCCrn	9.1	7.1	0.959	0.99	-8.5	10.1	18.4	0.722	0.66	11.7	
%CCrn	87.0	9.2				84.3	26.7				
dE1	24.7	13.5	0.886	0.48	16.2	13.0	10.2	0.882	0.61	12.4	*
%E1	145.2	20.9				124.3	18.9				**
dE4	18.8	12.6	0.887	0.53	17.7	7.3	9.8	0.879	0.68	13.8	*
%E4	77.8	11.1				91.0	13.1				**
dE9	20.8	12.9	0.886	0.52	17.1	9.2	9.9	0.879	0.66	13.4	*
%E9	135.5	19.3				116.0	17.8				**
dE10	20.8	12.0	0.887	0.46	18.0	10.1	10.0	0.822	0.75	6.8	**
%E10	140.0	19.6				119.5	18.6				**
dE11F	23.3	13.2	0.883	0.50	15.8	3.5	24.0	0.321	0.34	40.0	*
%E11F	141.6	19.3				112.1	35.9				

* $p<0.03$ ** $p<0.02$

Appendix C21 Fit v Frail Males - CCr Prediction Study - Mann-Whitney Test

	fit males (n=31)					frail males (n=31)					
	fit mean	fit s.d.	fit r	fit m	fit c	frail mean	frail s.d.	frail r	frail m	frail c	p
age (years)	78.0	6.9				77.9	6.9				
mobility score	1.2	0.5				3.6	0.6				****
weight (kg)	72.8	10.5				66.0	11.0				*
SA (m2)	1.86	0.16				1.76	0.16				*
urine volume (ml)	1546	472				1399	706				
UCr (mg in 24h)	1260	240				993	532				****
[SCr] (mg/100ml)	1.46	0.42				1.60	0.77				
24h CCr (ml/min)	64.9	19.5				49.2	25.3				**
24h CCr/SA (ml/min/1.73m2)	60.5	17.5				47.7	23.7				*
dCCram	-2.2	15.0	0.911	1.00	2.5	-17.5	27.5	0.667	1.01	17.2	*
%CCram	102.3	21.8				139.1	71.3				*
dCCrpm	-7.0	8.6	0.975	1.08	0.8	-3.1	21.9	0.787	1.13	-4.0	
%CCrpm	109.8	12.6				103.9	47.3				
dCCrn	6.1	9.3	0.961	0.97	-3.9	4.7	17.3	0.788	0.90	0.9	
%CCrn	90.0	17.9				92.3	30.2				
dE1	18.4	10.2	0.855	0.69	1.6	5.6	18.3	0.700	0.57	15.4	****
%E1	142.6	25.2				119.8	56.8				****
dE4	12.8	10.0	0.860	0.78	1.6	0.4	18.9	0.699	0.64	17.4	****
%E4	80.8	13.7				109.6	42.1				****
dE9	14.6	9.9	0.860	0.75	1.5	2.0	18.7	0.699	0.62	16.8	****
%E9	131.7	22.7				110.8	52.7				****
dE10	15.8	10.6	0.835	0.73	1.4	3.4	21.2	0.653	0.63	15.8	***
%E10	137.3	24.5				116.7	57.1				****
dE11F	8.5	15.6	0.747	0.89	-1.4	-9.4	27.9	0.540	0.68	25.3	****
%E11F	120.6	25.7				94.5	50.7				****

* p<0.05 ** p<0.01 *** p<0.001 **** p<0.0005

Appendix C22 Very Fit v Frail Males - CCr Prediction Study - Mann-Whitney Test

	very fit males (n=7)					frail males (n=7)					
	v.fit mean	v.fit s.d.	v.fit r	v.fit m	v.fit c	frail mean	frail s.d.	frail r	frail m	frail c	p
age (years)	69.3	3.4				69.1	3.3				
mobility score	1.0	0.0				3.4	0.5				**
weight (kg)	76.6	8.8				64.0	11.3				*
SA (m2)	1.91	0.15				1.76	0.16				
urine volume (ml)	1881	647				1675	645				
UCr (mg in 24h)	1519	145				1153	688				*
[SCr] (mg/100ml)	1.44	0.30				1.57	0.89				
24h CCr (ml/min)	76.0	15.5				58.4	29.3				
24h CCr/SA (ml/min/1.73m2)	68.7	12.7				57.9	32.2				
dCCram	-6.3	17.2	0.897	2.00	-62.5	-29.0	38.7	0.626	1.48	3.1	
%CCram	106.7	23.0				151.3	77.1				
dCCrpm	-3.0	11.3	0.516	0.45	40.7	11.5	15.4	0.891	1.34	-29.6	
%CCrpm	106.0	16.3				72.5	27.1				
dCCrn	8.3	8.0	0.808	0.88	-0.2	2.0	21.4	0.357	0.30	35.4	
%CCrn	87.8	10.3				104.3	37.2				
dE1	22.2	10.0	0.766	0.57	10.7	7.7	27.4	0.491	0.41	26.8	
%E1	143.0	23.0				129.9	83.3				
dE4	16.3	9.9	0.772	0.64	11.0	2.1	28.5	0.491	0.46	29.7	
%E4	79.2	12.3				116.9	71.6				
dE9	18.3	9.9	0.772	0.62	10.7	4.0	28.1	0.491	0.44	28.8	
%E9	133.3	21.2				121.2	77.6				
dE10	18.8	10.2	0.662	0.41	22.7	5.4	33.9	0.589	0.58	22.9	
%E10	137.7	22.2				127.1	84.0				
dE11F	20.5	8.4	0.851	0.62	8.5	-10.5	40.5	0.473	0.73	26.4	*
%E11F	137.6	15.1				108.0	72.3				

* $p<0.05$ ** $p<0.0001$

Appendix D1

INFORMATION SHEET FOR WATER TABLET STUDY

Dr. Parker is carrying out a project with the St. Marks Road GP's surgery, and the Research Institute for the Care of the Elderly, St. Martins Hospital, looking at the way in which the water tablets you are taking are handled by your body. Thank you for helping us with this study.

These instructions should be followed carefully during the period of the study. If you are not sure what to do at any time , then let someone know. Phone numbers are given at the bottom of this sheet.

1) Take your water tablets (Frumil or Frusemide) at your usual time and write the exact time on this paper.

2) After taking your tablets please empty your bladder (pass water) as soon as you possibly can. Throw this specimen away into the toilet.

3) Each and every time you pass a urine specimen after this time, please collect it, in a container provided. Write on the container the time at which the urine was collected. ALL urine should be collected, even at night time. If you should happen to run out of containers in which to collect urine, a clean jam jar, or similar, covered in silver foil or paper, will do.

4) Exactly 24 hours later from the time of taking your water tablets, collect a final urine specimen & save it

5) You may take your next water tablet once you have collected the final urine specimen.

6) Dr. Parker will call three times during the first day of the study, to take blood samples, (during the day time), and once again the following morning.

7) Please record how many times you eat meat during the study and approximately how much you eat.

8) Dr. Parker will ask you at what time you got up & went to bed on the study day, and how far you walked.

Very many thanks.

DAY URINE COLLECTION TO START.....

TIME WATER TABLET TAKEN.....

MEAT EATEN.....

IN CASE OF QUERY PLEASE DO NOT HESITATE TO CONTACT

SUE ELLMERS (STUDY CO-ORDINATOR) AT WORK ON BATH 835866 OR

AT HOME ON BATH 834963

Appendix D2

STUDY TO MEASURE FRUSEMIDE METABOLISM IN THE ELDERLY

Name : . . . . . Study No. . . . . .

Address : . . . . . Study Date. . . . . .

. . . . . .

. . . . . Mobility Score. . . .

. . . . . .

. . . . . Height/cm. . . . . .

Tel No. : . . . . . .

Weight/kg. . . . . .

D.O.B. : . . . . . Surface area/m^2 . . . .

Age : . . . . . .

G.P. : . . . . . .

Diagnoses : . . . . . .

. . . . . .

. . . . . .

. . . . . .

. . . . . .

Continent or catheterised

Drugs : . . . . . .

. . . . . .

. . . . . .

. . . . . .

. . . . . .

. . . . . .

Intended Time for FRU to be taken :

Time FRU taken : Dose FRU given :

		INTENDED	ACTUAL
Blood samples :	2h	.....-	.....
	4h	.....-	.....
	8h	.....-	.....
	24h	.....-	.....

		Time	Vol/ml
Urine samples :	1)	.....-	.....
	2)	.....-	.....
alcohol	3)	.....-	.....
	4)	.....-	.....
	5)	.....-	.....
tobacco	6)	.....-	.....
	7)	.....-	.....
	8)	.....-	.....
meat	9)	.....-	.....
	10)	.....-	.....
	11)	.....-	.....
	12)	.....-	.....
	13)	.....-	.....

TOTAL URINE VOL : .....IN .....HRS

Comments :

RICE, St. Martins Hospital, Bath. Tel : 834963

Appendix D3 Demographic Details of Subjects Taking Part in FRUSEMIDE Study

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F01GPF	F / 70	74/158	Y / 2	no	occ.	angina OA	Frumil,nifedipine,GTN atenolol, Mucaine
F04GPF	F / 85	51/153	Y / 1	no	occ.	asthma cataracts	Frumil,salbutamol inh. prednisolone,temazepam
F05GPF	F / 74	79/157	Y / 1	no	occ.	LVF, OA, nocturia	Frumil,Surgam,terodilene
F06GPF	F / 70	70/166	Y / 1	N/K	N/K	chest pain,IHD,SOB migraine, back pain	Frumil,temazepam Migravess,co-proxamol
F09GPF	F / 77	41/156	Y / 2	no	occ.	oedema diverticulitis L.thoracoplasty	Frumil,loperamide methocarbamol
F11GPF	F / 80	74/168	Y / 1	no	occ.	AF & hypertension goitre - euthyroid	Frumil,enalapril
F14GPF	F / 78	73/161	N / 3	no	no	IDDM, angina, CHF OA, hiatus hernia gout	frusemide,Actrapid,Monotard spironolactone,ranitidine quninine,codydramol,GTN
F18GPF	F / 79	45/155	Y / 1	3/day	no	MI, thyroidectomy	frusemide, folic acid
F21GPF	F / 74	66/162	Y / 1	N/K	N/K	IHD, hypertension	Frumil, atenolol
F22GPF	F / 67	41/148	Y / 1	no	N/K	CABG,mitral & aortic valve replacements angina	frusemide, digoxin warfarin
F24GPF	F / 82	56/161	N / 3	no	no	CCF, thoracoplasty # R. NOF '88	Frumil, nitrazepam
F26GPF	F / 78	75/152	Y / 2	no	occ.	COAD, hiatus hernia diverticulitis, OA	Frumil,prednisolone ranitidine,salbutamol neb.
F27GPF	F / 82	76/153	Y / 3	no	occ.	CCF, polymyalgia DVT, sinusitis	Frumil, prednisolone diazepam, Beconase
F28GPF	F / 81	41/152	N / 4	no	occ.	AF, R. hemiplegia # L humerus & femur	Frumil, digoxin paracetamol
F29GPF	F / 93	61/152	N / 3	no	occ.	cholecystectomy angina, OA	Frumil,isosorbide DN, GTN spray,chlormethiazole
F40F	F / 65	84/175	N / 4	no	no	MS, catheterised spastic paraparesis	Frumil,propranolol,senna terodilene,thioridazine
F47F	F / 81	72/154	N / 4	no	no	# NOF, osteoporosis anaemia	Frumil,ferrous sulphate
F49F	F / 81	84/168	N / 3	no	occ.	NIDDM, RA, angina MI '86 & '88 hypothyroid	frusemide,tolbutamide,GTN prednisolone,Milpar thyroxine,co-proxamol

Appendix D3 Demographic Details of Subjects Taking Part in FRUSEMIDE Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F50F	F / 100	38/152	N / 4	no	7/wk	osteoporosis	Frumil,Milpar,co-codamol
F51F	F / 67	36/157	N / 4	heavy	occ.	muscular dystrophy GU, heart failure	frusemide,spironolactone ranitidine
F52F	F / 95	50/139	N / 4	no	occ.	pleural effusion LVF	Frumil, GTN temazepam
F55F	F / 87	78/165	N / 4	no	no	CCF, COAD, OA chest infection	frusemide,captopril,digoxin prednisolone,isosorbide DN Becotide&salbutamol inh.
M02GPF	M / 86	92/169	N / 3	no	no	LVF, COAD, anxiety trigeminal neuralgia	Frumil, digoxin aminophylline
M03GPF	M / 82	71/158	N / 3	N/K	N/K	claudication, COAD hypertension	Frumil, Beconase salbutamol inh.
M07GPF	M / 78	88/172	Y / 2	N/K	N/K	angina,asthma,gout cataracts & myopia IHD, leg oedema	Frumil,GTN,prednisolone aminophylline,allopurinol
M08GPF	M / 78	69/172	Y / 1	N/K	N/K	cardiac murmur partial gastrectomy R. Dupytrons	Frumil,digoxin,Gaviscon nitrazepam,folic acid cytamen
M10GPF	M / 67	81/173	Y / 1	N/K	N/K	R leg ulcer cervical laminectomy	Frumil,verapamil,dothiepin carbamazepine,temazepam
M13GPF	M / 67	78/176	Y / 1	no	no	CHF, COAD, asthma blind L eye, gout	Frumil,prednisolone salbutamol&Atrovent nebs
M16GPF	M / 83	91/166	Y / 2	pipe	occ.	LVF, hypertension varicose leg ulcer	Frumil, oxprenolol
M17GPF	M / 84	93/175	N / 3	no	no	LVF, hypertension TURP, renal colic	Frumil,captopril,digoxin diclofenac,diazepam
M25GPF	M / 69	64/162	Y / 1	N/K	N/K	angina,LVF renal calculus diverticulitis	frusemide, captopril GTN, digoxin, aspirin
M45F	M / 69	70/185	N / 3	no	no	LVF, leg ulcers	frusemide, captopril paracetamol, senna
M48F	M / 89	64/170	N / 4	no	yes	LVF, peripheral vascular disease, below knee amputation	frusemide,captopril,Milpar morphine sulphate,thyroxine flavoxate,Trental
M53F	M / 76	63/173	N / 4	no	occ.	CVA '89 bladder instability	Frumil,baclofen,aspirin terodilene,co-proxamol Duovent & Bricanyl inh.
M54F	M / 88	49/175	N / 4	no	no	CVA '87, hypothyroid MI '82, # NOF	frusemide,sinemet,thyroxine prednisolone,pivampicillin

Appendix D4 FRUSEMIDE in Urine and Serum of Subjects in FRU Study

subject start	FRU dose mg	urine colln	T.int mins	[UFRU] mg/dl	urine vol ml	mg FRU in urine	cum mg UFRU	SFRU sample time	time from 0 hour	[SFRU] ncg/dl
F01GPF	40	0910	115	0.68	411	2.79	2.79	0915	2.00	31.33
@ 0715		1020	70	0.36	340	1.21	4.00	1112	3.95	17.67
		1121	61	0.45	154	0.69	4.69	1520	8.08	4.09
		1233	72	0.87	69	0.60	5.29	0718	24.05	1.11
		1402	89	1.15	53	0.61	5.90			
		1740	218	0.19	335	0.65	6.55			
		2000	140	0.07	290	0.19	6.74			
		2205	125	0.12	144	0.17	6.91			
		0430	385	0.01	720	0.04	6.95			
		0655	145	0.01	245	0.03	6.98			
M02GPF	80	0715	75	2.19	185	4.05	4.05	0755	1.92	115.93
@ 0600		0800	45	2.57	226	5.81	9.86	1000	4.00	41.07
		0915	75	11.84	76	9.00	18.86	1405	8.08	14.55
		1045	90	4.44	66	2.93	21.76	0605	24.08	4.28
		1230	105	3.55	62	2.20	23.99			
		1500	150	3.09	58	1.79	25.78			
		1700	120	1.93	45	0.87	26.65			
		2000	180	1.57	72	1.13	27.78			
		2400	240	0.96	90	0.86	28.64			
		0600	360	0.50	125	0.63	29.27			
M03GPF	80	0900	105	1.48	330	4.89	4.89	0915	2.00	129.68
@ 0715		1100	120	0.00	545	11.73	16.62	1120	4.08	67.08
		1215	75	2.27	385	8.74	25.36	1519	8.07	18.20
		1715	300	2.32	164	3.80	29.16	0715	24.00	3.87
		2300	345	1.83	134	2.45	31.61			
		0530	390	1.16	122	1.42	33.03			
		0720	110	1.00	8	0.08	33.11			
F04GPF	40	0845	75	1.58	60	0.95	0.95	0930	2.00	52.56
@ 0730		0955	70	1.24	266	3.29	4.24	1135	4.08	23.77
		1220	145	2.38	228	5.43	9.67	1535	8.08	12.09
		1625	245	3.07	83	2.55	12.22	0740	24.00	0.00
		1745	80	2.18	44	0.96	13.18			
		2120	215	1.81	80	1.45	14.63			
		0645	565	2.01	167	3.35	17.98			
F05GPF	40	0915	120	0.71	406	2.87	2.87	0915	2.00	29.00
@ 0715		1105	110	1.03	217	2.23	5.10	1120	4.08	34.97
		1335	150	1.00	228	2.28	7.38	1520	8.08	15.41
		1635	180	0.86	154	1.32	8.70	0715	24.00	6.50
		2115	280	0.49	237	1.15	9.85			
		0440	445	0.17	450	0.77	10.62			
		0645	125	0.08	220	0.17	10.79			
F06GPF	40	1147	107	0.61	432	2.65	2.65	1205	2.08	61.45
@ 1000		1346	119	1.07	628	6.73	9.38	1407	4.12	40.85
		1433	47	0.92	142	1.30	10.68	1800	8.00	7.17
		1643	130	0.57	430	2.44	13.12	0947	23.78	0.00
		1955	192	0.36	611	2.22	15.34			
		0600	605	0.39	230	0.89	16.23			
		0958	258	0.42	50	0.21	16.44			

Appendix D4 FRUSEMIDE in Urine and Serum of Subjects in FRU Study (contd)

subject start	FRU dose mg	urine colln	T.int mins	[UFRU] mg/dl	urine vol ml	mg FRU in urine	cum mg UFRU	SFRU sample time	time from 0 hour	[SFRU] mcg/dl
M07GPF	40	1020	75	0.93	108	1.00	1.00	1105	2.00	24.59
@ 0905		1110	50	1.08	130	1.41	2.41	1300	3.92	17.84
		1210	60	1.59	107	1.70	4.11	1720	8.25	3.42
		1310	60	1.39	108	1.50	5.61	1544	30.65	0.00
		1445	95	1.20	138	1.66	7.27			
		1615	90	0.70	150	1.05	8.32			
		2025	250	0.74	157	1.16	9.48			
		0030	245	0.25	165	0.41	9.89			
		0510	280	0.06	594	0.37	10.26			
		0900	230	0.04	318	0.13	10.39			
M08GPF	40	1100	150	0.83	223	1.84	1.84	1030	2.00	34.18
@ 0830		1600	300	1.74	212	3.68	5.52	1230	4.00	24.70
		2030	270	1.19	232	2.77	8.29	1630	8.00	14.77
		2315	165	0.77	88	0.68	8.97	0833	24.05	7.05
		0700	465	0.51	232	1.19	10.16			
		0829	89	0.48	60	0.29	10.45			
F09GPF	40	0845	85	1.72	339	5.82	5.82	0925	2.08	48.60
@ 0720		1130	165	3.81	155	5.91	11.73	1120	4.00	28.11
		1730	360	2.46	139	3.42	15.15	1520	8.00	13.49
		2330	360	0.52	174	0.91	16.06	0722	24.03	
		0430	300	0.15	265	0.40	16.46			
		0700	150	0.23	100	0.23	16.69			
M10GPF	40	2127	147	0.51	807	4.12	4.12	2110	2.17	42.06
@ 1900		2223	56	0.98	245	2.41	6.53	2245	3.75	39.91
		2343	80	1.19	248	2.95	9.48	0925	14.42	15.04
		0430	287	1.70	172	2.93	12.41	1905	24.08	16.25
		0948	318	1.62	97	1.57	13.98			
		1300	192	1.63	62	1.01	14.99			
		1448	108	0.54	156	0.84	15.83			
		1642	114	0.38	168	0.63	16.46			
		1908	146	0.89	120	1.07	17.53			
F11GPF	40	1000	120	1.15	435	5.01	5.01	1105	3.08	69.90
@ 0800		1145	105	1.99	486	9.65	14.66	1303	5.05	20.83
		1330	105	3.22	138	4.45	19.11	1605	8.08	1.06
		1545	135	3.32	74	2.46	21.57	0818	24.30	0.00
		1815	150	1.87	60	1.12	22.69			
		2130	195	0.86	122	1.05	23.74			
		0110	220	0.47	114	0.54	24.28			
		0530	260	0.05	110	0.06	24.34			
		0730	120	0.01	165	0.01	24.35			
M13GPF	40	0950	175	0.47	528	2.46	2.46	0857	2.03	55.09
@ 0655		1125	95	0.97	117	1.13	3.59	1058	4.05	19.66
		1345	140	0.82	107	0.88	4.47	1520	8.42	0.00
		1610	145	0.08	262	0.20	4.67	0705	24.17	0.00
		2205	355	0.04	375	0.15	4.82			
		0650	525	0.00	555	0.00	4.82			

Appendix D4 FRUSEMIDE in Urine and Serum of Subjects in FRU Study (contd)

subject start	FRU dose mg	urine colln	T.int mins	[UFRU] mg/dl	urine vol ml	mg FRU in urine	cum mg UFRU	SFRU sample time	time from 0 hour	[SFRU] mcg/dl
F14GPF	120	1120	140	0.45	825	3.73	3.73	1105	2.08	24.70
@ 0900		1154	34	1.13	546	6.15	9.88	1310	4.17	63.88
		1445	171	1.76	540	9.52	19.40	1719	8.32	30.77
		1610	85	0.98	387	3.81	23.21	0901	24.02	16.35
		1745	95	1.35	202	2.72	25.93			
		2400	375	1.62	265	4.29	30.22			
		0530	330	0.69	300	2.06	32.28			
		0855	205	0.52	152	0.79	33.07			
M16GPF	40	1100	180	0.35	552	1.91	1.91	1018	2.30	57.45
@ 0800		1200	60	0.44	174	0.76	2.67	1214	4.23	48.22
		1500	180	0.43	278	1.20	3.87	1558	7.97	12.23
		1830	210	0.35	267	0.94	4.81	0807	24.12	1.33
		2030	120	0.04	310	0.12	4.93			
		2300	150	0.06	250	0.15	5.08			
		0230	210	0.11	125	0.14	5.22			
		0700	270	0.04	200	0.08	5.30			
M17GPF	80	0945	45	0.24	127	0.30	0.30	1110	2.00	140.53
@ 0900		1255	190	0.47	309	1.45	1.75	1308	3.97	164.64
		1452	117	0.81	118	0.96	2.71	1652	7.78	67.64
		1727	155	0.97	79	0.77	3.48	0909	23.98	6.77
		1905	98	0.74	103	0.76	4.24			
		2230	205	0.41	326	1.35	5.59			
		0700	510	0.28	320	0.90	6.49			
		0839	99	0.06	174	0.11	6.60			
F18GPF	40	1015	135	0.68	167	1.13	0.13	0954	1.90	22.65
@ 0800		1117	62	0.79	220	1.74	2.87	1202	4.03	27.75
		1313	116	1.30	230	3.00	5.87	1605	8.08	9.71
		1546	153	1.27	172	2.18	8.05	0803	24.05	4.88
		2245	419	1.32	195	2.58	10.63			
		0620	455	0.59	152	0.90	11.53			
		0810	110	0.46	48	0.22	11.75			
F21GPF	40	0900	105	1.03	289	2.97	2.97	0915	2.00	59.86
@ 0715		1020	70	1.32	208	2.75	5.72	1150	4.58	26.11
		1330	90	0.77	224	1.73	7.45	1515	8.00	6.55
		1525	115	0.44	78	0.34	7.79	0725	24.17	0.00
		1900	215	0.39	120	0.47	8.26			
		2200	180	0.21	95	0.20	8.46			
		0545	465	0.00	393	0.00	8.46			
		0710	85	0.00	42	0.00	8.46			
F22GPF	40	0935	69	0.83	204	1.69	1.69	1030	2.07	78.54
@ 0826		1010	35	1.24	136	1.68	3.37	1247	4.35	104.91
		1220	130	1.48	242	3.57	6.97	1623	7.95	16.32
		1600	220	1.73	203	3.51	10.45	0822	24.93	7.91
		1900	180	0.89	130	1.16	11.61			
		2220	200	0.60	114	0.68	12.29			
		0800	580	0.39	64	0.25	12.54			

Appendix D4 FRUSEMIDE in Urine and Serum of Subjects in FRU Study (contd)

subject start	FRU dose mg	urine colln	T.int mins	[UFRU] mg/dl	urine vol ml	mg FRU in urine	cum mg UFRU	SFRU sample time	time from 0 hour	[SFRU] mcg/dl
F24GPF	40	1130	150	1.59	417	6.63	6.63	1100	2.00	38.54
@ 0900		1555	265	1.76	350	6.17	12.80	1314	4.23	23.04
		2135	340	1.32	44	0.58	13.38	1650	7.83	6.86
		0830	655	1.35	235	3.18	16.56	0903	24.05	3.67
M25GPF	40	1035	145	1.26	575	7.22	7.22	1022	2.20	24.98
@ 0810		1230	115	0.82	179	1.46	8.68	1216	4.10	9.51
		1445	135	1.61	75	1.21	9.89	1608	7.97	0.00
		1845	240	1.05	129	1.35	11.24	0800	23.83	0.00
		2230	225	0.55	203	1.12	12.36			
		0345	315	0.46	171	0.78	13.14			
		0745	240	0.44	126	0.55	13.69			
F26GPF	40	1000	90	0.72	235	1.69	1.69	1026	1.93	49.20
@ 0830		1045	45	1.78	330	5.87	7.56	1230	4.00	21.52
		1145	60	1.58	350	5.53	13.09	1630	8.00	0.00
		1430	165	2.08	240	5.00	18.09	0822	23.87	0.00
		0230	720	0.66	80	0.53	18.62			
		0545	195	1.02	128	1.31	19.93			
		0745	120	0.43	145	0.63	20.56			
F27GPF	40	0935	65	1.57	58	0.91	0.91	1043	2.22	103.93
@ 0830		1055	80	2.56	130	3.33	4.24	1247	4.28	131.31
		1230	95	2.72	250	6.79	11.03	1655	8.42	19.05
		1515	165	2.49	205	5.10	16.13	0838	24.13	0.00
		2330	495	0.66	140	0.92	17.05			
		0545	375	1.13	160	1.80	18.85			
		0805	140	0.05	80	0.04	18.89			
F28GPF	40	1000	120	2.48	168	4.17	4.17	1000	2.00	114.00
@ 0800		1155	115	4.27	238	10.16	14.33	1153	3.88	107.86
		1610	255	5.26	110	5.79	20.12	1606	8.10	44.86
		2200	350	0.00	255	0.00	20.12	0755	23.92	4.88
		0230	270	0.00	190	0.00	20.12			
		0800	330	0.00	320	0.00	20.12			
F29GPF	40	1010	130	1.04	48	0.50	0.50	0948	1.80	24.42
@ 0800		1145	95	1.65	210	3.47	3.97	1202	4.03	46.24
		1340	115	2.77	315	8.73	12.70	1555	7.92	30.73
		2100	440	4.46	225	10.04	22.74	0732	23.53	0.00
		0115	255	2.33	135	3.14	25.88			
		0740	385	1.01	175	1.76	27.64			
F40F	40	1130	255	0.93	870	8.11	8.11	0930	2.25	51.85
@ 0715		1640	310	2.53	120	3.03	11.14	1130	4.25	34.00
		2015	215	3.83	90	3.45	14.59	1530	8.25	8.83
		2400	225	2.58	180	4.65	19.24	0700	23.75	0.00
		0400	240	0.33	350	1.14	20.38			
		0715	195	0.34	270	0.91	21.29			

Appendix D4 FRUSEMIDE in Urine and Serum of Subjects in FRU Study (contd)

subject start	FRU dose mg	urine colln	T.int mins	[UFRU] mg/dl	urine vol ml	mg FRU in urine	cum mg UFRU	SFRU sample time	time from 0 hour	[SFRU] mcg/dl
M45F	80	0900	130	0.99	350	3.47	3.47	0930	2.67	32.57
@ 0650		1130	150	1.38	324	4.47	7.94	1137	4.79	19.09
		1445	195	1.95	116	2.26	10.20	1540	8.84	8.64
		2245	480	0.92	182	1.68	11.88	0700	24.17	2.00
		0247	242	0.21	590	1.23	13.11			
		0630	223	0.49	655	3.21	16.32			
		0715	45	0.34	96	0.33	16.65			
F47F	80	1245	58	0.93	415	3.87	3.87	1337	1.83	45.39
@ 1147		1400	75	1.98	375	7.41	11.28	1600	4.22	22.06
		1700	180	2.58	220	5.67	16.95	2000	8.22	4.38
		2230	330	2.78	110	3.06	20.01	1125	23.37	1.67
		0620	470	1.43	215	3.07	23.08			
		0945	205	1.19	115	1.37	24.45			
		1210	145	0.98	90	0.88	25.33			
M48F	40	1115	210	1.83	200	3.66	3.66	0955	2.17	127.35
@ 0745		1635	320	2.69	160	4.30	7.96	1145	4.00	92.01
		0815	940	1.55	548	8.49	16.45	2140	13.92	38.92
								0720	23.58	26.00
F49F	40	1300	180	1.69	108	1.83	1.83	1155	1.92	70.10
@ 1000		1400	60	1.80	20	0.36	2.19	1615	6.25	0.00
		1520	80	1.15	80	0.92	3.11	2145	11.75	0.00
		1645	85	0.48	62	0.30	3.41	0715	21.25	0.00
		2000	195	0.00	50	0.00	3.41			
		2130	90	0.00	18	0.00	3.41			
		0225	295	0.00	310	0.00	3.41			
		0550	205	0.00	178	0.00	3.41			
		0950	240	0.00	14	0.00	3.41			
F50F	40	0900	120	1.63	110	1.79	1.79	0945	2.75	108.00
@ 0700		1115	135	1.62	102	1.65	3.44	1140	4.67	73.80
		1615	300	3.11	30	0.93	4.37	2130	14.50	0.00
		0015	480	1.50	30	0.45	4.82	0700	24.00	0.00
		0700	405	0.39	200	0.77	5.59			
F51F	40	0855	100	2.52	410	10.33	10.33	0945	2.50	93.36
@ 0715		1025	90	1.57	225	3.54	13.87	1150	4.58	56.96
		1120	55	2.85	75	2.14	16.01	1620	9.08	13.80
		1200	40	0.23	225	0.52	16.53	0655	23.67	8.51
		1340	100	4.61	80	3.69	20.22			
		1720	220	2.66	110	2.93	23.15			
		0630	790	1.07	260	2.77	25.92			
F52F	40	1120	195	2.46	188	4.62	4.62	0955	1.83	210.92
@ 0805		1900	460	2.56	75	1.92	6.54	1130	3.42	230.24
		2230	210	2.80	70	1.96	8.50	2120	13.25	27.74
		0230	240	2.22	130	2.88	11.38	0742	23.62	11.92
		0610	220	1.69	86	1.45	12.83			
		0750	100	1.69	36	0.61	13.44			

Appendix D4 FRUSEMIDE in Urine and Serum of Subjects in FRU Study (contd)

subject start	FRU dose mg	urine colln	T.int mins	[UFRU] mg/dl	urine vol ml	mg FRU in urine	cum mg UFRU	SFRU sample time	time from 0 hour	[SFRU] mcg/dl
M53F	40	1000	105	1.11	340	3.77	3.77	1035	2.33	138.94
@ 0815		1155	115	4.36	360	15.69	19.46	1210	3.92	56.48
		1600	245	4.69	210	9.85	29.31	1635	8.33	13.79
		2220	380	2.48	255	6.33	35.64	0754	23.65	0.00
		0540	440	1.39	370	5.14	40.78			
M54F	40	1020	140	1.88	60	1.13	1.13	1015	2.25	49.09
@ 0800		1155	95	2.69	90	2.42	3.55	1200	4.00	109.53
		1615	260	3.34	144	4.81	8.36	1615	8.25	65.75
		2025	250	3.49	70	2.44	10.80	0733	23.55	41.54
		2300	155	1.70	90	1.53	12.33			
		0300	240	1.42	78	1.11	13.44			
		0736	276	1.08	124	1.34	14.78			
F55F	80	1145	135	2.42	235	5.69	5.69	1145	2.25	213.22
@ 0930		1400	135	2.56	230	5.89	11.58	1405	4.58	117.33
		2055	415	3.18	240	7.63	19.21	1705	7.58	71.05
								0905	23.58	52.56

Appendix D5 SCr, UCr, & CCr, calculated over 8 & 24 hour periods, for Subjects in FRUSEMIDE Study

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F01GPF	1.16	1402	6.78	122.4	11.92	1027	26		43		
		2205	8.05	486.5	63.26	769	87		145		
		0655	8.83	374.0	38.76	965	61		102		
			23.66	982.9	35.60	2761		60		1.83	57
M02GPF	1.41	1500	9.00	438.3	65.12	673	58		94		
		2400	9.00	505.5	244.19	207	66		106		
		0600	6.00	319.3	255.42	125	63		102		
			24.00	1263.0	125.67	1005		62		2.10	51
M03GPF	1.56	1215	5.00	227.9	18.09	1260	49		153		
		2300	10.75	350.4	117.60	298	35		109		
		0720	8.33	140.5	108.10	130	18		56		
			24.08	718.9	42.59	1688		32		1.79	31
F04GPF	1.00	1220	4.83	145.6	26.28	554	50		114		
		2120	9.00	222.5	107.51	207	41		93		
		0645	9.42	249.8	149.58	167	44		100		
			23.25	617.9	66.58	928		44		1.49	51
F05GPF	1.70	1335	6.33	265.6	31.21	851	41		75		
		2115	7.67	440.6	112.69	391	56		102		
		0645	9.50	611.4	91.26	670	63		115		
			23.50	1317.8	68.92	1912		55		1.88	50
F06GPF	1.03	1643	6.72	325.6	19.95	1632	78		147		
		1955	3.20	150.2	24.58	611	76		143		
		0958	14.05	304.3	108.67	280	35		66		
			23.97	780.1	30.92	2523		53		1.81	50
M07GPF	1.75	1615	7.17	479.6	64.73	741	64		110		
		0030	8.25	520.5	161.66	322	60		103		
		0900	8.50	465.0	50.99	912	52		90		
			23.92	1465.3	74.19	1975		58		2.07	49
M08GPF	2.70	1600	7.50	303.5	69.76	435	25		86		
		2315	7.25	360.1	112.54	320	31		107		
		0829	9.23	463.1	158.60	292	31		107		
			23.98	1126.6	107.6	1047		29		1.83	27
F09GPF	1.15	1130	4.17	142.9	28.93	494	50		106		
		2330	12.00	349.9	111.78	313	42		89		
		0700	7.50	281.5	77.12	365	54		115		
			23.67	774.3	66.07	1172		47		1.34	61
M10GPF	1.32	2343	4.72	307.8	23.68	1300	82		106		
		0948	10.08	580.7	215.88	269	73		95		
			9.33	585.2	115.66	506	79		103		
			24.13	1473.9	71.03	2075		77		1.99	67
F11GPF	1.46	1545	7.75	340.9	30.09	1133	50		94		
		2130	5.75	320.7	176.19	182	64		121		
		0730	10.00	430.9	110.76	389	49		92		
			23.50	1092.4	64.11	1704		53		1.88	49

Appendix D5 SCr, UCr, & CCr, calculated over 8 & 24h periods, for Subjects in FRUSEMIDE Study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M13GPF	1.30	1345	6.83	310.7	41.31	752	58		81		
		2205	8.33	632.3	99.27	637	97		135		
		0650	8.75	407.9	73.49	555	60		83		
			23.91	1350.9	69.49	1944		72		1.96	64
F14GPF	1.95	1445	5.75	334.4	17.50	1911	50		109		
		2400	9.25	486.0	56.91	854	45		98		
		0855	8.92	462.8	102.38	452	44		96		
			23.92	1282.9	39.88	3217		46		1.83	43
M16GPF	1.59	1500	7.00	423.3	42.16	1004	63		91		
		2300	8.00	568.5	68.74	827	74		107		
		0700	8.00	532.1	163.72	325	70		101		
			23.00	1523.9	70.68	2156		69		2.08	58
M17GPF	4.19	1457	5.95	387.8	70.00	554	26		130		
		2230	7.55	357.9	70.45	508	19		95		
		0839	10.15	425.5	86.14	494	17		85		
			23.65	1171.2	75.27	1556		20		2.15	16
F18GPF	1.11	1546	7.77	242.5	30.74	789	47		104		
		2245	6.98	222.0	113.87	195	48		107		
		0810	9.42	267.7	133.86	200	43		96		
			24.17	732.3	61.85	1184		45		1.40	56
F21GPF	1.59	1330	6.25	305.3	42.35	721	51		102		
		2200	8.50	419.2	143.06	293	52		104		
		0710	9.17	427.2	98.20	435	49		98		
			23.92	1151.7	79.48	1449		50		1.74	50
F22GPF	1.41	1600	7.57	272.8	34.75	785	43		165		
		2220	6.33	185.7	76.10	244	35		135		
		0800	9.67	62.5	97.72	64	8		31		
			23.57	521.1	47.68	1093		26		1.41	34
F24GPF	1.28	1555	6.92	473.9	61.79	767	89		178		
		2135	5.67	47.3	107.53	44	11		22		
		0830	10.92	382.3	162.70	235	46		92		
			23.50	903.6	86.39	1046		50		1.60	54
M25GPF	1.60	1445	6.58	274.6	33.12	829	43		86		
		2230	7.75	428.0	128.91	332	58		116		
		0745	9.25	433.9	146.08	297	49		98		
			23.58	1136.4	77.94	1458		50		1.71	51
F26GPF	1.62	1430	6.00	168.3	14.57	1155	29		100		
		0230	12.00	135.1	168.90	80	12		41		
		0745	5.25	357.6	130.98	273	70		241		
			23.25	661.0	43.83	1508		29		1.81	28
F27GPF	3.34	1515	6.75	238.2	37.04	643	18		129		
		2330	8.25	214.8	153.44	140	13		93		
		0805	8.58	199.7	83.19	240	12		86		
			23.58	653.0	63.83	1023		14		1.83	13

Appendix D5 SCr, UCr, & CCr, calculated over 8 & 24h periods, for Subjects in FRUSEMIDE Study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F28GPF	2.77	1610	8.17	83.1	16.10	516	6		50		
		2200	5.83	150.2	58.90	255	16		108		
		0800	10.00	263.4	51.65	510	16		108		
			24.00	496.4	38.75	1281		12		1.33	16
F29GPF	1.44	1340	5.67	110.8	19.34	573	23		85		
		2100	7.33	148.3	65.93	225	23		85		
		0740	10.67	293.8	94.78	310	32		119		
			23.67	553.0	49.91	1108		27		1.63	29
F40F	1.77	1130	4.25	135.0	15.52	870	30		97		
		2015	8.75	214.0	101.90	210	23		74		
		0715	11.00	450.0	56.25	800	39		126		
			24.00	799.0	42.50	1880		31		2.15	25
M45F	3.20	1445	7.75	408.0	51.65	790	27		96		
		2245	8.00	245.0	134.62	182	16		57		
		0715	8.50	658.0	49.07	1341	40		142		
			24.25	1311.0	56.68	2313		28		1.90	25
F47F	1.43	1700	5.21	183.0	18.12	1010	41		124		
		2230	5.50	108.0	98.18	110	23		70		
		1210	13.67	396.0	94.29	420	34		103		
			24.38	687.0	44.61	1540		33		1.72	33
M48F	1.66	1115	3.50	49.2	24.62	200	14		61		
		1635	5.33	132.6	82.89	160	25		109		
		0815	15.67	383.8	70.03	548	25		109		
			24.50	566.0	62.33	908		23		1.75	23
F49F	0.72	1645	6.75	209.9	77.75	270	72		131		
		2130	4.75	103.8	152.66	68	51		93		
		0950	12.33	336.2	66.98	502	63		115		
			23.83	650.0	77.38	840		55		2.00	48
F50F	3.34	1615	4.25	57.4	191.35	30	7		58		
		0700	14.75	442.0	192.17	230	15		125		
		1115	4.25	74.2	72.78	102	9		75		
			23.25	573.0	158.29	362		12		1.28	17
F51F	1.11	1340	6.42	391.7	38.59	1015	92		263		
		1720	3.67	45.4	41.25	110	19		54		
		0630	13.16	115.0	44.24	260	13		37		
			23.25	552.1	39.86	1385		35		1.26	49
F52F	2.91	1120	3.25	31.5	16.73	188	6		67		
		2230	11.17	111.5	76.90	145	6		67		
		0750	9.33	222.3	88.23	252	14		156		
			23.75	365.0	62.39	585		9		1.41	11
M53F	1.98	1600	8.00	797.8	87.67	910	84		350		
		2220	7.33	223.9	87.79	255	26		108		
		1020	12.00	380.4	78.92	482	25		113		
			27.33	1420.0	86.22	1647		24		1.54	27

Appendix D5 SCr, UCr, & CCr, calculated over 8 & 24h periods, for Subjects in FRUSEMIDE Study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M54F	1.78	1615	8.25	251.3	85.49	294	29		63		
		2300	6.75	183.3	114.59	160	25		54		
		0736	8.60	236.8	117.21	202	26		57		
			23.60	671.0	102.29	656		46		1.54	51
F55F	1.81	1400	4.50	261.9	56.33	465	54				
		2055	6.92	333.9	139.14	240	44				
			11.42	596.0	84.54	705				1.91	

Appendix D6 Summary of Kinetic Parameters Studied in Subjects Taking FRUMIL or FRUSEMIDE

subject		FRU mg	urine vol ml	% Du/ dose	t1/2 h	Cls ml/min	Cls/kg ml/min /kg	Clr ml/min	Clr 0-6h ml/min	UCr mg/24h	[SCr] mg/dl	CCr ml/min	CCr/SA ml/min 1.73m2
F01GPF	FM	40	2761	17.45	4.898	191.6	2.589	66.9	139.4	983	1.16	60	57
F04GPF	FM	40	928	44.95	4.097	120.8	2.369	108.6	158.9	618	1.00	44	51
F05GPF	FM	40	1912	26.98	9.132	72.9	0.923	39.4	83.0	1318	1.70	55	51
F06GPF	FM	40	2523	41.10	1.855	119.1	1.701	97.9	260.5	780	1.03	53	51
F09GPF	FM	40	1172	41.73	3.278	122.6	2.990	102.3	158.9	774	1.15	47	61
F11GPF	FM	40	1704	60.88	0.823	144.3	1.950	175.7	*	1092	1.46	53	49
F14GPF	FS	120	3217	27.56	11.416	104.8	1.436	57.8	119.6	1283	1.95	46	43
F18GPF	FS	40	1184	29.38	9.330	99.5	2.211	58.5	114.4	732	1.11	45	56
F21GPF	FM	40	1449	21.15	1.724	137.2	2.079	58.0	149.4	1152	1.59	50	50
F22GPF	FS	40	1093	31.35	6.953	39.0	0.951	24.4	59.6	521	1.41	26	32
F24GPF	FM	40	1046	41.40	7.291	116.6	2.082	96.5	196.4	904	1.28	50	54
F26GPF	FM	40	1508	51.40	1.733	191.6	2.555	196.9	520.2	661	1.62	29	28
F27GPF	FM	40	1023	47.23	1.488	47.0	0.618	44.4	95.7	653	3.34	14	13
F28GPF	FM	40	1281	50.30	4.607	31.1	0.759	31.3	80.6	496	2.77	12	16
F29GPF	FM	40	1108	69.10	6.594	61.4	1.007	84.8	96.0	553	1.44	27	29
F40F	FM	40	1880	53.23	2.051	130.3	1.401	138.6	93.5	799	1.77	31	25
F47F	FM	80	1540	31.63	4.943	278.9	4.358	176.4	322.7	687	1.43	33	33
F49F	FS	40	840	8.55	*	*	*	*	*	650	0.72	55	48
F50F	FM	40	472	13.98	3.494	47.8	1.258	13.4	16.8	573	3.34	12	16
F51F	FS	40	1385	64.80	6.637	49.3	1.369	63.4	129.0	552	1.11	35	48
F52F	FM	40	585	33.60	8.504	15.5	0.310	10.3	37.8	365	2.91	9	11
F55F	FS	80	*	*	20.166	19.3	0.247	*	43.7	*	1.81	*	*
M02GPF	FM	80	1005	36.59	6.776	115.0	1.250	84.1	255.3	1263	1.41	62	51
M03GPF	FM	80	1688	41.39	5.344	93.8	1.321	77.6	201.3	719	1.56	32	31
M07GPF	FM	40	1975	25.98	2.114	273.2	3.105	141.9	379.2	1465	1.75	58	48
M08GPF	FM	40	1047	26.13	11.897	71.2	1.032	37.2	68.9	1127	2.70	29	27
M10GPF	FM	40	2075	43.83	7.557	58.8	0.726	51.5	91.7	1474	1.32	77	67
M13GPF	FM	40	1944	12.05	1.361	196.1	2.514	47.3	227.3	1351	1.30	72	64
M16GPF	FM	40	2156	13.25	4.105	83.8	0.921	22.2	42.4	1524	1.59	69	57
M17GPF	FM	80	1556	8.25	4.497	58.0	0.624	9.6	7.2	1171	4.19	20	16
M25GPF	FS	40	1458	34.23	1.361	421.9	6.592	288.8	*	1136	1.60	50	51
M45F	FS	80	2313	20.81	6.267	262.5	3.750	109.3	185.9	1310	3.20	28	25
M48F	FS	40	908	41.13	10.719	19.7	0.308	16.1	19.7	566	1.66	23	23
M53F	FM	80	1535	50.98	2.168	129.5	2.641	132.0	399.1	1420	1.78	46	52
M54F	FS	40	656	36.95	15.595	14.3	0.227	10.6	21.5	671	1.98	24	27

Appendix D7 FRUSEMIDE v FRUMIL - Mann-Whitney test

	FM n	FS n	FM min	FS min	FM max	FS max	FM Q1	FS Q1	FM Q3	FS Q3
age (years)	25	10	65	67	100	89	74	69	84	87
Mobility score	25	10	1	1	4	4	1	1	4	4
UCr (mg)	25	9	365	521	1524	1310	657	559	1291	1209
SCr (mg/100ml)	25	10	1.00	0.72	4.19	3.20	1.31	1.11	2.24	1.96
CCr (ml/min)	25	9	9	23	77	55	28	25	57	48
CCr/SA (ml/min/1.73m2)	25	9	11	23	67	56	26	26	53	49
FRU dose (mg)	25	10	40	40	80	120	40	40	40	80
%Du/dose	25	9	8.25	8.55	69.10	33.07	23.56	12.15	48.76	21.28
Urine vol (ml)	25	9	472	656	2761	3217	1046	874	1928	1886
weight (kg)	25	10	38	36	93	84	54	44	80	74
SA (m2)	25	10	1.28	1.26	2.15	2.00	1.57	1.38	1.98	1.90
t1/2 (h)	25	9	0.823	1.360	11.897	20.170	1.953	6.450	6.685	13.51
Cls (ml/min)	25	9	15.5	14.3	278.9	421.9	60.1	19.5	140.7	183.6
Cls/kg (ml/min/kg)	25	9	0.310	0.227	4.358	6.592	0.922	0.278	2.534	2.981
Clr (ml/min)	25	8	9.6	10.6	196.9	288.8	38.3	18.2	120.3	97.9
Cl 0-6h (ml/min)	24	8	7.2	19.7	520.2	185.9	81.2	27.0	248.3	126.7

Appendix D7 FRUSEMIDE v FRUMIL - Mann-Whitney test (contd)

	FM median	FS median	FM mean	FS mean	FM s.d.	FS s.d.	p
age (years)	80.0	78.5	79.6	77.4	8.5	8.9	
Mobility score	2.0	3.0	2.4	2.8	1.2	1.3	
UCr (mg)	904	671	957	825	351	324	
SCr (mg/100ml)	1.590	1.630	1.864	1.655	0.839	0.678	
CCr (ml/min)	46.0	35.0	41.8	36.9	19.8	12.3	
CCr/SA (ml/min/1.73m2)	48.8	43.5	40.3	39.2	17.6	12.4	
FRU dose (mg)	40.0	40.0	48.0	56.0	16.3	28.0	
%Du/dose	41.10	31.35	36.18	32.75	16.17	15.35	
Urine vol (ml)	1535	1184	1515	1450	564	819	
weight (kg)	71.0	64.0	68.8	61.8	16.9	16.1	
SA (m2)	1.810	1.730	1.770	1.661	0.261	0.267	
t1/2 (h)	4.105	9.330	4.493	9.830	2.849	5.550	**
Cls (ml/min)	116.6	49.3	116.3	114.5	68.5	139.1	
Cls/kg (ml/min/kg)	1.401	1.369	1.723	1.899	0.988	2.092	
Clr (ml/min)	77.6	58.2	81.8	78.7	54.5	90.8	
Cl 0-6h (ml/min)	144.4	87.0	170.1	86.7	131.3	59.5	

** $p<0.01$

Appendix D8 Female v Male Elderly taking FRUMIL - Mann-Whitney test

	Female n	Male n	Female min	Male min	Female max	Male max	Female Q1	Male Q1	Female Q3	Male Q3
age (years)	16	9	65	67	100	86	74	72	84	84
Mobility score	16	9	1	1	4	4	1	1	4	3
UCr (mg)	16	9	365	719	1318	1524	584	1149	963	1470
SCr (mg/100ml)	16	9	1.00	1.30	3.34	4.19	1.19	1.37	2.52	2.24
CCr (ml/min)	16	9	9	20	60	77	17	31	52	71
CCr/SA (ml/min/1.73m2)	16	9	11	16	61	67	18	29	51	60
FRU dose (mg)	16	9	40	40	80	80	40	40	40	80
%Du/dose	16	9	13.98	8.25	69.10	50.97	28.14	12.65	51.12	42.61
Urine vol (ml)	16	9	472	1005	2761	2156	1029	1291	1836	2025
weight (kg)	16	9	38	49	93	93	50	70	75	92
SA (m2)	16	9	1.28	1.54	2.15	2.15	1.43	1.81	1.83	2.09
t1/2 (h)	16	9	0.823	1.360	9.132	11.900	1.763	2.140	6.181	7.170
Cls (ml/min)	16	9	15.5	58.0	278.9	273.2	51.2	65.0	142.5	162.8
Cls/kg (ml/min/kg)	16	9	0.310	0.624	4.358	3.105	0.944	0.823	2.508	2.577
Clr (ml/min)	16	9	10.3	9.6	196.9	141.9	40.7	29.7	131.1	108.1
Clr/kg (ml/min/kg)	16	9	0.206	0.103	2.756	2.694	0.629	0.392	2.313	1.353
Cl 0-6h (ml/min)	15	9	16.8	7.2	520.2	399.1	83.0	55.7	196.4	317.3

Appendix D8 Female v Male Elderly taking FRUMIL - Mann-Whitney test (contd)

	Female median	Male median	Female mean	Male mean	Female s.d.	Male s.d.	p
age (years)	80.5	78.0	80.5	77.9	9.4	7.0	
Mobility score	2.5	2.0	2.5	2.2	1.3	1.1	
UCr (mg)	730.5	1351.0	775.5	1279.3	257.5	251.6	**
SCr (mg/100ml)	1.525	1.590	1.812	1.956	0.805	0.938	
CCr (ml/min)	38.5	58.0	36.2	51.7	17.6	20.7	
CCr/SA (ml/min/1.73m2)	41.0	51.1	37.1	46.0	17.4	17.4	
FRU dose (mg)	40.0	40.0	42.5	57.8	10.0	21.1	
%Du/dose	41.56	26.12	40.38	28.71	15.48	15.4	
Urine vol. (ml)	1365	1688	1431	1665	626	423	
weight (kg)	65.0	81.0	63.1	79.1	15.8	14.4	*
SA (m2)	1.730	1.990	1.671	1.946	0.245	0.195	*
t1/2 (h)	3.796	4.500	4.157	5.090	2.605	3.332	
Cls (ml/min)	119.9	93.8	114.3	119.9	68.8	71.9	
Cls/kg (ml/min/kg)	1.826	1.250	1.809	1.570	1.040	0.927	
Clr (ml/min)	90.7	51.5	90.1	67.0	58.4	46.2	
Clr/kg (ml/min/kg)	1.394	0.636	1.411	0.938	0.859	0.799	
Cl 0-6h (ml/min)	139.4	201.3	160.7	185.8	127.8	143.5	

* $p<0.03$ ** $p<0.001$

Appendix D9 Female v Male Elderly taking FRUSEMIDE - Mann-Whitney test

	Female n	Male n	Female min	Male min	Female max	Male max	Female Q1	Male Q1	Female Q3	Male Q3
age (years)	6	4	67	69	87	89	67	69	83	89
Mobility score	6	4	1	1	4	4	1	2	4	4
UCr (mg)	5	4	521	566	1283	1310	536	592	1008	1266
SCr (mg/100ml)	6	4	0.72	1.60	1.95	3.20	1.01	1.62	1.85	2.90
CCr (ml/min)	5	4	26	23	55	50	31	23	51	45
CCr/SA (ml/min/1.73m2)	5	4	32	23	56	51	38	23	52	45
FRU dose (mg)	6	4	40	40	120	80	40	40	90	70
%Du/dose	5	4	8.55	20.81	64.80	41.13	18.05	24.17	48.08	40.08
Urine vol (ml)	5	4	840	656	3217	2313	967	719	2301	2099
weight (kg)	6	4	36	63	84	70	40	63	80	69
SA (m2)	6	4	1.26	1.54	2.00	1.90	1.30	1.58	1.93	1.86
t1/2 (h)	5	4	6.64	1.36	20.17	15.60	6.80	2.59	15.79	14.38
Cls (ml/min)	5	4	19.3	14.3	104.8	421.9	29.1	15.7	102.2	382.0
Cls/kg (ml/min/kg)	5	4	0.247	0.230	2.211	6.590	0.599	0.250	1.823	5.880
Clr (ml/min)	4	4	24.4	10.6	63.8	288.8	32.8	12.0	62.5	243.9
Cl 0-6h (ml/min)	5	3	43.7	19.7	129.0	185.9	51.7	19.7	124.3	185.9
Clr/kg (ml/min/kg)	4	4	0.60	0.17	1.77	4.51	0.64	0.19	1.65	3.77

Appendix D9 Female v Male Elderly taking FRUSEMIDE - Mann-Whitney test (contd)

	Female median	Male median	Female mean	Male mean	Female s.d.	Male s.d.	p
age (years)	78.5	78.5	76.5	78.8	8.0	11.3	
Mobility score	3.0	3.5	2.7	3.0	1.4	1.4	
UCr (mg)	650	904	748	921	311	359	
SCr (mg/100ml)	1.260	1.820	1.352	2.110	0.466	0.746	
CCr (ml/min)	45.0	26.0	41.4	31.3	11.2	12.7	
CCr/SA (ml/min/1.73m2)	47.6	26.2	45.3	31.4	8.7	12.9	
FRU dose (mg)	40.0	40.0	60.0	50.0	33.5	20.0	
%Du/dose	29.37	35.59	32.33	33.28	20.32	8.78	
Urine vol (ml)	1184	1183	1544	1334	956	734	
weight (kg)	59.0	64.0	59.5	65.3	21.1	3.2	
SA (m2)	1.615	1.730	1.618	1.725	0.331	0.148	
t1/2 (h)	9.33	8.49	10.90	8.49	5.53	6.09	
Cls (ml/min)	49.3	141.1	62.4	179.6	37.9	198.7	
Cls/kg (ml/min/kg)	1.37	2.03	1.24	2.72	0.72	3.06	
Clr (ml/min)	58.2	62.7	51.1	106.2	18.0	129.9	
Cl 0-6h (ml/min)	114.4	21.5	93.3	75.7	38.8	95.4	
Clr/kg (ml/min/kg)	1.046	0.91	1.115	1.62	0.529	2.03	

Appendix D10 Results From All Subjects Taking FRUMIL

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	25	65	100	74	84	80.0	79.6	8.5
Mobility score	25	1	4	1	4	2.0	2.4	1.2
UCr (mg)	25	365	1524	657	1290	904.0	956.9	351.4
SCr (mg/100ml)	25	1.00	4.19	1.31	2.24	1.590	1.864	0.839
CCr (ml/min)	25	9	77	28	57	46.0	41.8	19.8
CCr/SA (ml/min/1.73m2)	25	11	67	26	53	48.8	40.3	17.6
FRU dose (mg)	25	40	80	40	40	40.0	48.0	16.3
%Du/dose	25	8.25	69.10	23.56	48.76	41.1	36.2	16.2
Urine vol (ml)	25	472	2761	1046	1928	1535	1515	564
weight (kg)	25	38	93	54	80	71.0	68.8	16.9
SA (m2)	25	1.28	2.15	1.57	1.98	1.810	1.770	0.261
t1/2 (h)	25	0.823	11.897	1.953	6.685	4.105	4.493	2.849
AUC (mg/l/h)	25	1.22	21.65	2.41	6.39	3.980	5.102	4.377
Cls (ml/min)	25	15.5	278.9	60.1	140.7	116.6	116.3	68.5
Cls/kg (ml/min/kg)	25	0.310	4.358	0.922	2.534	1.401	1.723	0.988
Clr (ml/min)	25	9.6	196.9	38.3	120.3	77.6	81.8	54.5
Clr/kg (ml/min/kg)	25	0.103	2.756	0.562	1.926	0.914	1.241	0.853
Clr 0-6h (ml/min)	24	7.2	520.2	81.2	248.3	144.4	170.1	131.3

Appendix D11 Spearmans Coefficient of Rank Correlation for Subjects taking FRUMIL

	age years	M.Sc	UCr mg	[SCr] mg/dl	CCr ml/min	CCr/SA 1.73m2	FRU mg	%Du/ dose	urine vol ml	weight kg	SA m2	t1/2 h	Cls ml/min	Cls/kg ml/min /kg
n	25	25	25	25	25	25	25	25	25	25	25	25	25	25
MSc	0.441													
UCr	0.468	0.439												
SCr	0.279	0.483	0.108											
CCr	0.507	0.601 ***	0.784 ****	0.632 ***										
CCr/SA	0.432	0.517	0.604 ***	0.775 ****	0.871 ****									
mg FRU	0.229	0.424	0.153	0.111	0.056	0.055								
% Du/ dose	0.031	0.172	0.393	0.095	0.256	0.142	0.097							
U vol	0.685 ****	0.387	0.600 ***	0.265	0.640 ***	0.442	0.042	0.208						
weight	0.278	0.263	0.609 ***	0.063	0.458	0.099	0.118	0.218	0.549 ***					
SA	0.323	0.308	0.687 ****	0.048	0.506	0.148	0.118	0.242	0.573 ***	0.979 ****				
t1/2	0.294	0.152	0.032	0.012	0.066	0.021	0.180	0.134	0.195	0.090	0.090			
Cls	0.506	0.271	0.317	0.488	0.503	0.449	0.069	0.026	0.436	0.164	0.196	0.472		
Cls/kg	0.359	0.113	0.127	0.499	0.329	0.443	0.097	0.021	0.200	0.202	0.169	0.373	0.896 ****	
Clr	0.298	0.065	0.005	0.466	0.222	0.245	0.097	0.549 ***	0.161	0.011	0.009	0.373	0.763 ****	0.760 ****

*** p<0.01 **** p<0.001

Appendix D12 Results From All Subjects Taking FRUSEMIDE

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	10	67	89	69	87	78.5	77.4	8.9
Mobility score	10	1	4	1	4	3.0	2.8	1.3
UCr (mg)	9	521	1310	559	1209	671	825	324
SCr (mg/100ml)	10	0.72	3.20	1.11	1.96	1.630	1.655	0.678
CCr (ml/min)	9	23	55	25	48	35.0	36.9	12.3
CCr/SA (ml/min/1.73m2)	9	23	56	26	49	43.5	39.2	12.4
FRU dose (mg)	10	40	120	40	80	40.0	56.0	28.0
%Du/dose	9	8.55	64.80	24.19	39.04	31.35	32.75	15.35
Urine vol (ml)	9	656	3217	874	1886	1184	1450	819
weight (kg)	10	36	84	44	74	64.0	61.8	16.1
SA (m2)	10	1.26	2.00	1.38	1.90	1.730	1.661	0.267
t1/2 (h)	9	1.360	20.170	6.450	13.510	9.330	9.830	5.550
AUC (mg/l/h)	9	0.79	34.44	2.94	20.16	8.55	11.81	11.13
Cls (ml/min)	9	14.3	421.9	19.5	183.6	49.3	114.5	139.1
Cls/kg (ml/min/kg)	9	0.227	6.592	0.278	2.981	1.369	1.899	2.092
Clr (ml/min)	8	10.6	288.8	18.2	97.9	58.2	78.7	90.8
Clr/kg (ml/min/kg)	8	0.168	4.512	0.337	1.720	1.046	1.369	1.399
Clr 0-6h (ml/min)	8	19.7	185.9	27.0	126.7	87.0	86.7	59.5

Appendix D13 Spearmans Coefficient of Rank Correlation for Subjects Taking FRUSEMIDE

	age years	MSc	UCr mg	SCr mg/dl	CCr ml/min	CCr/SA 1.73m2	FRU mg	%Du/ dose	urine vol ml	weight kg	SA m2	t1/2 h	Cls ml/min	Cls/kg ml/min /kg
n	10	10	9	10	9	9	10	9	9	10	10	9	9	9
M.Sc	0.507													
UCr	0.067	0.235												
SCr	0.229	0.306	0.579											
CCr	0.284	0.494	0.350	0.456										
CCr/SA	0.381	0.518	0.050	0.652	0.733									
mg FRU	0.011	0.131	0.701	0.631	0.146	0.263								
% Du/ dose	0.045	0.510	0.517	0.022	0.550	0.067	0.496							
U vol	0.591	0.332	0.600	0.282	0.350	0.217	0.730	0.217						
weight	0.450	0.119	0.531	0.232	0.476	0.195	0.564	0.718	0.094					
SA	0.470	0.161	0.513	0.261	0.373	0.296	0.539	0.715	0.047	0.985 ****				
t1/2	0.726	0.543	0.111	0.301	0.429	0.332	0.297	0.095	0.492	0.377	0.333			
Cls	0.596	0.656	0.639	0.075	0.819	0.450	0.216	0.557	0.900 ***	0.100	0.067	0.767		
Cls/kg	0.581	0.702	0.602	0.167	0.797	0.524	0.108	0.535	0.820	0.017	0.000	0.800	0.983 ****	
Clr	0.641	0.450	0.452	0.182	0.738	0.533	0.195	0.286	0.754	0.120	0.119	0.881 ***	0.907 ***	0.929 ***

*** p<0.01 **** p<0.001

Appendix D14 Fit v Frail Elderly taking FRUMIL - Mann-Whitney U test

	fit min	frail min	fit max	frail max	fit Q1	frail Q1	fit Q3	frail Q3
age (years)	67	65	85	100	70.8	81.0	80.5	93.0
Mobility score	1	3	3	4	1.0	3.0	2.0	4.0
UCr (mg)	618	365	1524	1420	745.8	553.0	1379.5	1171.0
SCr (mg/100ml)	1.00	1.28	3.34	4.19	1.16	1.43	1.71	2.91
CCr (ml/min)	14	9	77	62	40.3	12.0	62.3	46.0
CCr/SA (ml/min/1.73m2)	13	11	67	54	43.3	16.1	58.2	51.1
FRU dose (mg)	40	40	40	80	40.0	40.0	40.0	80.0
%Du/dose	12.1	8.3	60.9	69.1	20.2	31.6	45.5	51.0
Urine vol (ml)	928	472	2761	1880	1141	1005	2095	1556
weight (kg)	41	38	91	93	68.3	49.0	79.5	92.0
SA (m2)	1.34	1.28	2.08	2.15	1.79	1.41	1.97	2.10
t1/2 (h)	0.823	2.051	11.897	8.504	1.665	3.494	5.563	6.776
Cls (ml/min)	47.0	15.5	273.2	278.9	72.5	47.8	191.6	129.5
Cls/kg (ml/min/kg)	0.618	0.310	3.105	4.358	0.922	0.759	2.563	2.082
Clr (ml/min)	22.2	9.6	196.9	176.4	43.2	13.4	116.9	132.0
Clr/kg (ml/min/kg)	0.244	0.103	2.625	2.756	0.573	0.353	2.191	1.723

n = 14 for fit subjects n = 11 for frail subjects

Appendix D14 Fit v Frail Elderly taking FRUMIL - Mann-Whitney U test (contd)

	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	77.5	82.0	76.0	84.1	5.7	9.6	**
Mobility score	1.0	4.0	1.5	3.5	0.7	0.5	***
UCr (mg))	1109.5	719.0	1069.4	814.0	328.3	340.0	
SCr (mg/100ml)	1.525	1.770	1.622	2.171	0.652	0.974	
CCr (ml/min)	53.0	31.0	50.7	30.4	17.5	17.0	**
CCr/SA (ml/min/1.73m2)	50.6	28.7	48.1	30.3	15.2	15.8	*
FRU dose (mg)	40.0	40.0	40.0	58.2	0.0	20.9	
%Du/dose	34.04	41.39	33.86	39.13	15.33	17.47	
Urine vol (ml)	1808	1281	1727	1245	567	449	
weight (kg)	74.5	61.0	72.4	64.4	13.2	20.5	
SA (m2)	1.830	1.630	1.824	1.700	0.202	0.318	
t1/2 (h)	2.696	4.943	4.004	5.115	3.334	2.064	
Cls (ml/min)	121.7	93.8	130.7	98.0	64.3	72.2	
Cls/kg (ml/min/kg)	2.014	1.258	1.862	1.546	0.872	1.137	
Clr (ml/min)	62.5	84.1	85.0	77.7	54.5	56.8	
Clr/kg (ml/min/kg)	0.891	1.093	1.252	1.226	0.842	0.907	

* $p<0.05$ ** $p<0.02$ *** $p<0.001$

Appendix D15 Fit v Frail Elderly taking FRUSEMIDE - Mann-Whitney test

	fit n	frail n	fit min	frail min	fit max	frail max	fit Q1	frail Q1	fit Q3	frail Q3
age (years)	3	7	67	67	79	89	67	69	79	88
Mobility score	3	7	1	3	1	4	1	3	1	4
UCr (mg)	3	6	521	552	1136	1310	521	563	1136	1290
SCr (mg/100ml)	3	7	1.11	0.72	1.60	3.20	1.11	1.22	1.60	1.98
CCr (ml/min)	3	6	26	23	50	55	26	24	50	48
CCr/SA (ml/min/1.73m2)	3	6	32	23	56	48	32	25	56	48
FRU dose (mg)	3	7	40	40	40	120	40	40	40	80
%Du/dose	3	6	29.37	8.55	34.22	64.80	29.37	17.75	34.22	47.04
Urine vol (ml)	3	6	1093	656	1458	3217	1093	794	1458	2539
weight (kg)	3	7	41	36	64	84	41	63	64	78
SA (m2)	3	7	1.31	1.26	1.71	2.00	1.31	1.54	1.71	1.91
t1/2 (h)	3	6	1.36	6.27	9.33	20.17	1.36	6.54	9.33	16.74
Cls (ml/min)	3	6	39.0	14.3	422.0	262.5	39.0	18.0	422.0	144.2
Cls/kg (ml/min/kg)	3	6	0.95	0.23	6.59	3.75	0.95	0.24	6.59	2.01
Clr (ml/min)	3	5	24.4	10.6	288.8	109.3	24.4	13.4	288.8	86.6
Clr/kg (ml/min/kg)	3	5	0.60	0.17	4.51	1.77	0.60	0.21	4.51	1.67
Cl 0-6h (ml/min)	2	6	59.6	19.7	114.4	185.9	59.6	21.0	114.4	143.2

Appendix D15 Fit v Frail Elderly taking FRUSEMIDE - Mann-Whitney test (contd)

	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	69.0	81.0	71.7	79.9	6.4	9.0	
Mobility score	1.0	4.0	1.0	3.6	0.0	0.5	*
UCr (mg)	732	660	796	839	313	358	
SCr (mg/100ml)	1.410	1.810	1.373	1.776	0.247	0.782	
CCr (ml/min)	45.0	31.5	40.3	35.2	12.7	12.9	
CCr/SA (ml/min/1.73m2)	50.6	35.2	46.0	35.7	12.5	11.9	
FRU dose (mg)	40.0	40.0	40.0	62.9	0.0	31.5	
%Du/dose	31.35	32.25	31.65	33.30	2.44	19.33	
Urine vol (ml)	1184	1147	1245	1553	190	1010	
weight (kg)	45.0	70.0	50.0	66.9	12.3	15.5	
SA (m2)	1.400	1.830	1.473	1.741	0.210	0.258	
t1/2 (h)	6.95	11.07	5.88	11.80	4.09	5.35	
Cls (ml/min)	100.0	34.5	187.0	78.3	206.0	96.4	
Cls/kg (ml/min/kg)	2.21	0.84	3.25	1.22	2.96	1.36	
Clr (ml/min)	58.5	57.8	123.9	51.5	143.8	40.2	
Clr/kg (ml/min/kg)	1.30	0.79	2.14	0.91	2.09	0.74	
Cl 0-6h (ml/min)	87.0	81.7	87.0	86.6	38.7	68.3	

* $p<0.0001$

Appendix D16 Fit v Frail Age Matched Elderly taking FRUMIL - Mann-Whitney test

	fit n	frail n	fit min	frail min	fit max	frail max	fit Q1	frail Q1	fit Q3	frail Q3
age (years)	8	7	77	76	85	86	78	81	83	84
Mobility score	8	7	1	3	3	4	1	3	2	4
UCr (mg)	8	7	618	496	1524	1420	655	687	1381	1263
SCr (mg/100ml)	8	7	1.00	1.28	3.34	4.19	1.23	1.41	2.46	2.77
CCr (ml/min)	8	7	14	12	69	62	29	20	57	50
CCr/SA (ml/min/1.73m2)	8	7	13	16	61	54	27	16	56	52
FRU dose (mg)	8	7	40	40	40	80	40	40	40	80
%Du/dose	8	7	13.25	8.25	60.88	50.97	26.01	31.62	50.36	50.30
U vol (ml)	8	7	928	1005	2156	1688	1029	1046	1907	1556
weight (kg)	8	7	41	41	91	93	56	49	85	92
SA (m2)	8	7	1.34	1.33	2.08	2.15	1.57	1.54	2.02	2.10
t1/2 (h)	8	7	0.82	2.17	11.90	7.29	1.55	4.50	4.10	6.78
Cls (ml/min)	8	7	47.0	31.1	273.2	278.9	74.3	58.0	179.8	129.5
Cls/kg (ml/min/kg)	8	7	0.618	0.624	3.105	4.358	0.949	0.759	2.881	2.641
Clr (ml/min)	8	7	22.2	9.6	196.9	176.4	39.0	31.3	167.3	132.0
Clr/kg (ml/min/kg)	8	7	0.244	0.103	2.625	2.756	0.550	0.763	2.465	2.694
Clr 0-6h (ml/min)	7	7	42.4	7.2	520.2	399.1	68.9	80.6	379.2	322.7

Appendix D16 Fit v Frail Age Matched Elderly taking FRUMIL - Mann-Whitney test (contd)

	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	79.0	82.0	80.1	81.7	2.9	3.1	
mobility score	2.0	3.0	1.8	3.4	0.7	0.5	**
UCr (mg)	933	904	989	951	368	341	
SCr (mg/100ml)	1.605	1.560	1.826	2.060	0.796	1.064	
CCr (ml/min)	45.5	33.0	42.9	36.4	17.9	17.5	
CCr/SA (ml/min/1.73m2)	48.6	33.2	41.9	36.1	16.9	16.6	
FRU dose (mg)	40.0	80.0	40.0	68.6	0.0	19.5	
%Du/dose	43.34	41.39	38.94	37.22	15.77	14.53	
U vol (ml)	1340	1535	1439	1379	468	270	
weight (kg)	74.5	64.0	70.6	66.6	17.1	20.2	
SA (m2)	1.830	1.720	1.791	1.747	0.258	0.297	
t1/2 (h)	2.70	4.94	3.69	5.09	3.53	1.68	*
Cls (ml/min)	121.7	115.0	131.8	117.6	72.8	79.3	
Cls/kg (ml/min/kg)	2.159	1.321	1.942	1.862	0.973	1.310	
Clr (ml/min)	105.4	84.1	103.7	86.8	65.4	56.7	
Clr/kg (ml/min/kg)	1.871	1.093	1.575	1.435	0.980	1.002	
Clr 0-6h (ml/min)	158.9	201.3	203.5	208.9	178.3	134.6	

* $p<0.05$ ** $p<0.005$

Appendix E1

INFORMATION SHEET FOR HEART TABLET STUDY

Dr. Parker is carrying out a project with St. Mark's Road Surgery looking at the way in which the heart tablets (Digoxin or Lanoxin) you take are handled by your body. This will involve noting the exact time at which your heart tablet was taken on 2 occasions, collecting your urine for 24 hours, and having 3 blood samples taken over 5 days. YOUR MEDICATION WILL NOT BE ALTERED OR STOPPED DURING THE TRIAL.

These instructions should be followed carefully during the study. If you are not sure what to do at any time, then let someone know. Phone numbers are given at the bottom of the page. If you change your mind and no longer want to help us, also phone these numbers.

1) On the morning of the study please do not take your heart tablet until Dr. Parker calls. When he arrives, he will ask you to empty your bladder. Following this, you may then take your heart tablet.

2) Every time you pass urine after taking the tablet please collect it all, in a separate container provided. Write on the container your name, and time at which the urine was passed. ALL urine should be collected, even at night time. If you should happen to run out of containers in which to collect urine, a clean jam jar, or similar, will do.

3) Eleven hours after taking your heart tablet Dr Parker will call at your home, and take a small blood sample from you. You must continue to collect your urine.

4) Exactly 24 hours from when you started the urine collection, you must again pass urine, and save it. Dr Parker will again call on you, take a blood sample, and collect the urine. Once the urine collection has been completed, you may take your heart tablet as usual.

5) About 5 days later, a further blood sample will be taken Dr. Parker will arrange this with you at the time of the study. We will need to know the exact time you took your heart tablet, but no urine collections will be necessary.

6) If you are taking any other tablets, you may take these as normal, but let us know what you have taken.

Dr. Parker will call at..... on.....
Please do not take your heart tablet until he arrives.

Dr. Parker will then call at..... on .....evening

and again at.....on.....morning
Please do not take your next heart tablet until he arrives.

We will phone you to arrange the last visit, which will be about five days later.

IN CASE OF QUERY, PLEASE DO NOT HESITATE TO CONTACT
SUE ELLMERS (STUDY CO-ORDINATOR) AT WORK ON BATH 835866
OR AT HOME ON BATH 834963

Appendix E2

MEASUREMENT OF DIGOXIN CLEARANCE IN THE ELDERLY

Name : . . . . . Study No. . . . . .

Address : . . . . . Study Date. . . . . .

. . . . . .

. . . . . Mobility Score . . . .

. . . . . .

. . . . . Height/cm. . . . . .

Tel No. . . . . .

Weight/kg. . . . . .

DOB : . . . . . .

Age : . . . . . Surface area/m^2. . . .

GP : . . . . . .

continent/catheterised

Diagnoses : . . . . . .

. . . . . .

. . . . . .

. . . . . .

. . . . . .

. . . . . .

Drugs : . . . . . .

. . . . . .

. . . . . .

. . . . . .

. . . . . .

Int. Start (1)..... (2)..... Date (1)..... (2).....

Act.Time n (1)..... (2)..... Date (1)..... (2).....

Pulse Rate (1).....(2).....Rhythm.....BP.....

Dose..... Dose unchanged 3/52..... Compliance Y / N

Digitalisation : sub-therapeutic - toxic - OK (from obs)

Blood Samples : Time/11h Date Time/11h Date

Intended : . . . . . .

Actual : . . . . . .

Start urine colln.....Date.....(discard urine)

Urine Samples : Time Vol/ml Combined

1) . . . . .-. . . . . .}. . . . . .

2) . . . . .-. . . . . .}. . . . . .

alcohol 3) . . . . .-. . . . . .}. . . . . .

4) . . . . .-. . . . . .}. . . . . .

5) . . . . .-. . . . . .}. . . . . .

tobacco 6) . . . . .-. . . . . .}. . . . . .

7) . . . . .-. . . . . .}. . . . . .

8) . . . . .-. . . . . .}. . . . . .

meat 9) . . . . .-. . . . . .}. . . . . .

10) . . . . .-. . . . . .}. . . . . .

11) . . . . .-. . . . . .}. . . . . .

12) . . . . .-. . . . . .}. . . . . .

TOTAL URINE VOL : . . . . . IN . . . . . HRS

Comments :

Appendix E3 Demographic Details and Medications of Subjects Taking Part in DIGOXIN Study

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F01D	F / 77	35/137	Y / 2	yes	occ.	AF, confusion # L. NOF '89	digoxin, paracetamol
F02D	F / 79	76/178	Y / 2	no	occ.	AF CCF & SOB	digoxin,prednisolone bendrofluazide,temazepam
F04D	F / 81	65/174	N / 5	3/day	7/wk	cellulitis L leg thyroidectomy depression catheterised	digoxin,paracetamol calcuim heparin inj. temazepam
F05D	F / 90	45/165	N / 5	no	no	decreased mobility catheterised	digoxin,spironolactone frusemide,paracetamol,Milpar
F08DGP	F / 81	41/152	N / 4	no	14/wk	CCF, fast AF CVA, catheterised # L femur, PE 1984	digoxin,co-amilofruse bisacodyl
F11D	F / 75	50/151	N / 4	no	no	fast AF, LVF, CVA	digoxin, trimethoprim
F12DGP	F / 79	51/151	Y / 1	no	14/wk	LVF, OA, piles inguinal hernia	digoxin,GTN,Aldactide co-proxamol,Epogam
F14DGP	F / 90	52/151	Y / 1	no	1/wk	glaucoma	digoxin
F15DGP	F / 82	54/156	Y / 2	no	no	AF, diverticulitis	digoxin
F16DGP	F / 78	60/152	Y / 1	no	occ.	CCF, mild asthma cystitis	digoxin,Navidrex K,GTN salbutamol inh.
F17DGP	F / 74	60/155	N / 3	no	4/wk	AF, COAD, OA, THR hypothyroid	digoxin, Aldactide thyroxine
F18DGP	F / 73	48/164	Y / 1	no	occ.	mitral incompetance LV hypertrophy AF, TIA '87	digoxin,warfarin,Dyazide verapamil
F19DGP	F / 76	75/165	Y / 1	no	14/wk	AF, OA spinal decompression	digoxin,nifedipine,amiloride diclofenac,frusemide
F20D	F / 85	61/149	N / 3	no	no	AF, angina, CCF NIDDM, blind	digoxin,co-amilofruse glibenclamide,co-codaprin
F21DGP	F / 90	45/155	N / 4	no	no	AF, CVA 1978 hypothyroid	digoxin, aspirin diazepam, co-amilofruse
F22D	F / 83	59/150	N / 3	no	occ.	AF, gout, OA, THR varicose ulcers	digoxin, bumetanide morphine sulphate,lactulose
F24DGP	F / 96	44/148	N / 3	no	occ.	AF,partially sighted	digoxin, co-amilofruse
F29D	F / 90	46/149	N / 4	no	no	AF, CCF, CVA 1982	digoxin, co-amilofruse
F30D	F / 81	53/152	N / 5	no	no	AF,CCF,IHD,leg ulcer Ca L breast & mets	digoxin,captopril,cefadroxil frusemide, vits B & C inj.

Appendix E3 Demographic Details and Medications of Subjects Taking Part in DIGOXIN Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F33D	F / 76	47/153	N / 3	no	occ.	AF, LVF, COAD thyrotoxicosis 1977	digoxin, co-amilofruse chlormethiazole,salbutamol
F34DGP	F / 83	58/174	N / 5	no	no	AF, R. THR CVA & L hemiplegia	digoxin, co-amilofruse temazepam
F37DGP	F / 82	51/145	Y / 2	no	no	CVA 1972, anaemia mitral valve disease	digoxin,warfarin,FeSO4 tetrabenazine,co-amilofruse
F44D	F / 77	43/160	N / 3	occ.	14/wk	cervical spondylosis CVA & R hemiplegia AF, depression	digoxin,Premarin lofepramine,thioridazine lactulose,paracetamol
F46DGP	F / 84	54/159	Y / 1	no	occ.	AF hypothyroidism	digoxin,bendrofluazide thyroxine,Slow K
F52DGP	F / 84	62/165	Y / 2	no	no	AF	digoxin,frusemide,nebumetone
F54DGP	F / 84	66/153	Y / 2	no	occ.	AF, chest pain cervical spondylosis	digoxin,diclofenac,Gaviscon captopril,bumetanide
M03D	M / 79	75/178	N / 4	no	occ.	CCF, MI 1977, SOB emphysema, HH	digoxin,co-amilofruse,Milpar salbutamol neb,prednisolone lofepramine,co-dydramol cefadroxil,temazepam
M06D	M / 79	62/168	N / 4	ex.	7/wk	LVF, OA, THR cellulitis L leg Ca prostate	digoxin, frusemide diclofenac, quinine flucloxacillin, Milpar
M09DGP	M / 66	96/178	Y / 1	no	occ.	UC; colostomy AF, CCF	digoxin,bendrofluazide GTN spray
M10DGP	M / 84	64/179	N / 3	ex.	7/wk	AF, CCF, emphysema R nephrectomy 1947	digoxin, co-amilozide GTN, Mucaine, oxygen
M13DGP	M / 79	75/173	Y / 2	occ. pipe	7/wk	AF, MI 1987 & 1989 LVF, PE 1970	digoxin, nitrazepam co-amilofruse
M23D	M / 68	80/187	Y / 1	no	no	cardiomyopathy 1989 thyrotoxicosis 1989 fast AF, CCF, TIA's	digoxin,frusemide,captopril warfarin,captopril co-dydramol
M25D	M / 87	41/162	N / 4	no	occ.	AF, pseudomembranous colitis 1990, COAD erythema multiforma	digoxin, theophylline Duovent inh., temazepam
M26D	M / 79	85/165	N / 3	no	occ.	COAD, OA	digoxin, co-amilofruse salbutamol inh., temazepam
M31DGP	M / 79	64/183	N / 3	no	21/wk	OA, bowel resection depression ? alcoholic	digoxin, temazepam paracetamol, senna ispagula husk

Appendix E3 Demographic Details and Medications of Subjects Taking Part in DIGOXIN Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
M35DGP	M / 89	60/169	Y / 1	no	no	AF, cholecystectomy prostatectomy	digoxin
M36DGP	M / 70	78/184	Y / 1	no	21/wk	prostatectomy post-op arrest AF, CVA x 2	digoxin, aspirin ascorbic acid,
M38DGP	M / 80	61/165	Y / 1	no	no	angina, MI 1976 hypertension TURP, claudication	digoxin,nifedipine,GTN isosorbide dinitrate inosital nicotinate
M40DGP	M / 75	78/179	Y / 1	no	occ.	fast AF,IHD,mitral stenotic murmur	digoxin
M41DGP	M / 85	71/178	Y / 1	no	no	angina cataracts	digoxin,oxprenolol,GTN naftidrofuryl oxalate
M42DGP	M / 82	69/171	Y / 1	no	no	CVA mitral valve disease	digoxin,Aldactide,aspirin ranitidine,Gaviscon quinine sulphate,lofepramine prochlorperazine inosital oxalate
M43DGP	M / 78	75/172	Y / 1	no	no	mild CCF, AF glaucoma	digoxin pilocarpine & timolol ED
M47DGP	M / 88	80/162	N / 3	no	no	AF,CCF,gangrene toe	digoxin, co-amilofruse
M48DGP	M / 78	74/162	Y / 1	no	21/wk	nephrectomy '42 (TB) urinary frequency	digoxin,bendrofluazide allopurinol,aminophylline diclofenac
M49D	M / 81	67/182	N / 4	no	occ.	AF, RA, leg ulcers urinary retention	digoxin,co-amilofruse ibuprofen,penicillin V indoramine
M50DGP	M / 81	77/165	N / 3	no	occ.	COAD cor pulmonale	digoxin,co-amilofruse prednisolone,gliclazide salbutamol & Pulmicort inh. erythromycin
M51DGP	M / 76	95/178	Y / 1	no	occ.	AF & IHD	digoxin
M53DGP	M / 67	65/171	N / 3	no	no	AF, angina, COAD indigestion	digoxin,co-amilofruse theophylline,prednisolone isosorbide MN,ranitidine salbutamol & terbutaline neb
M55DGP	M / 67	55/171	N / 3	ex.	occ.	COAD, MI 1990 ex. heavy smoker	digoxin,co-amilofruse,GTN prednisolone,salbutamol nebs isosorbide DN,theophylline ranitidine,temazepam

ft = fit: Y for fit, N for frail
EtOH = alcohol consumption- approx. units per week
occ. = occasional alcohol consumption

Appendix E4 Summary of Results from the DIGOXIN Study

No.	M.Sc	fit	SCr mg/dl	[UCr] mg/dl	time hours	CCr ml/min	U vol ml	U DIG mcg/24h	U DIG mcg/dl	S DIG ng/ml	dose mcg	% Du	total Cl ml/min	renal Cl ml/min
1	2	Y	0.62							0.50	125.0		116.32	
2	2	Y	1.27	65.5	25.42	69.6	2059	51.77	2.51	0.83	62.5	82.83	35.04	43.31
4	5	N	2.69	420.8	23.83	63.5	580	23.80	4.10	0.80	125.0	19.04	72.70	20.66
5	5	N	2.19							3.00	125.0		19.39	
8	4	N	2.38	95.9	23.15	29.8	1026	23.90	2.33	0.39	62.5	38.24	74.56	42.56
11	4	N	0.36	85.6	23.92	141.2	852	50.70	5.95	0.26	125.0	40.56	223.69	135.42
12	1	Y	0.58	75.8	24.00	84.9	936	43.42	4.64	0.48	125.0	34.74	121.17	62.82
14	1	Y	0.59	38.3	24.00	78.7	1746	49.39	2.83	0.20	62.5	79.02	145.40	171.49
15	2	Y	0.62	108.9	24.05	108.1	888	52.89	5.96	0.46	125.0	42.31	126.43	79.85
16	1	Y	0.72	44.5	24.07	80.1	1871	28.65	1.53	0.14	62.5	45.84	207.71	142.11
17	3	N	1.05	98.7	23.93	68.7	1050	77.96	7.42	0.32	125.0	62.37	181.75	169.18
18	1	Y	0.64	61.5	24.00	73.5	1102	43.05	3.91	0.28	62.5	68.88	103.86	106.77
19	1	Y	1.27	58.4	24.05	64.1	2010	97.74	4.86	1.08	250.0	39.10	107.70	62.85
20	3	N	0.82	66.9	23.91	44.1	776	74.53	9.60	1.74	250.0	29.81	66.85	29.75
21	4	N	1.18	100.4	24.50	28.4	490	23.74	4.84	0.68	62.5	37.98	42.76	24.24
22	3	N	1.18	45.3	23.50	31.3	1150	41.31	3.59	0.50	125.0	33.05	116.32	57.37
24	3	N	0.72	32.5	24.00	31.3	1000	52.00	5.20	2.50	250.0	20.80	46.53	14.44
29	4	N	0.69	71.9	24.25	35.1	490	78.00	15.92	1.31	125.0	62.40	44.40	41.35
30	5	N	2.32	38.8	24.33	9.4	820	19.40	2.37	1.38	62.5	31.04	21.07	9.76
33	3	N	0.88	45.1	24.83	27.0	786	72.70	9.25	1.03	125.0	58.16	56.47	49.02
34	5	N	0.60	57.7	24.00	61.6	922	86.90	9.43	1.00	125.0	69.52	58.16	60.35
37	2	Y	0.67	32.9	24.00	49.4	1449	63.31	4.37	0.88	250.0	25.32	132.18	49.96
44	3	N	0.34	74.3	25.34	126.6	881	165.90	18.83	1.24	250.0	66.36	93.81	92.91
46	1	Y	0.75	63.4	24.03	78.3	1336	39.80	2.98	0.53	125.0	31.84	109.74	52.15
52	2	Y	1.61	84.6	24.00	63.9	1752	85.10	4.86	1.85	250.0	34.04	62.88	31.94
54	2	Y	1.66	101.5	24.00	52.0	1224	20.90	1.71	0.36	62.5	33.44	80.78	40.32
3	4	N	2.08	115.6	27.00	77.9	2270	46.96	2.07	0.30	62.5	75.14	96.93	108.70
6	4	N	2.37	114.3	24.01	45.6	1362	67.12	4.93	1.70	125.0	53.70	34.21	27.42
9	1	Y	1.18	133.4	24.50	106.0	1378	90.00	6.53	0.52	250.0	36.00	223.69	120.19
10	3	N	1.16	91.0	25.04	44.6	854	44.70	5.23	0.59	125.0	35.76	98.58	52.61
13	2	Y	1.05	85.8	24.00	83.2	1466	62.00	4.23	0.49	125.0	49.60	118.69	87.87
23	1	Y	0.55	49.3	24.00	193.3	3105	117.80	3.79	0.45	250.0	47.12	258.49	181.79
25	4	N	0.73	49.3	24.00	28.8	614	22.80	3.71	0.49	62.5	36.48	59.35	32.31
26	3	N	0.98	104.0	25.25	108.4	1548	106.10	6.85	0.38	125.0	84.88	153.05	193.90
31	3	N	0.80	139.8	23.50	61.0	492	62.50	12.70	0.71	250.0	25.00	163.83	61.13
35	1	Y	0.91	87.7	23.92	84.9	1264	52.70	4.17	0.28	125.0	42.16	207.71	130.70
36	1	Y	1.45	222.4	23.28	87.2	794	72.60	9.14	0.44	250.0	29.04	264.36	114.58
38	1	Y	1.10	106.3	24.58	82.4	1258	32.70	2.60	0.26	62.5	52.32	111.85	87.34
40	1	Y	0.92	116.0	23.83	99.5	1128	88.90	7.88	0.76	125.0	71.12	76.53	81.23
41	1	Y	0.61	72.9	24.00	105.1	1266	52.90	4.18	0.64	125.0	42.32	90.87	57.40
42	1	Y	1.41	101.9	24.15	68.0	1364	61.90	4.54	1.08	125.0	49.52	53.85	39.80
43	1	Y	1.09	59.0	24.05	88.4	2356	64.10	2.72	0.50	250.0	25.64	232.64	89.03
47	3	N	1.41	60.8	24.22	46.7	1574	25.60	1.63	0.31	62.5	40.96	93.81	57.35
48	1	Y	1.28	68.4	23.83	56.1	1502	58.40	3.89	1.30	125.0	46.72	44.74	31.20
49	4	N	0.95	38.5	24.00	48.0	1707	54.00	3.16	0.72	125.0	43.20	80.78	52.08
50	3	N	0.91	30.7	24.25	86.8	3742	86.40	2.31	0.99	125.0	69.12	58.75	60.61
51	1	Y	1.16	104.6	24.00	102.3	1634	125.90	7.71	0.75	250.0	50.36	155.09	116.57
53	3	N	1.13	93.0	23.28	82.8	1406	163.90	11.66	0.98	250.0	65.56	118.69	116.14
55	3	N	0.77	38.1	24.00	96.0	2795	32.60	1.17	0.41	125.0	26.08	141.85	55.22

Appendix E5 SCr, UCr & CCr, calculated over 8 & 24 hour periods, for Subjects in DIGOXIN Study

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F02D	1.27	1515	6.75	348.7	37.7	925	68		97		
		1845	3.50	145.6	34.7	420	55		79		
		0955	15.17	853.5	119.5	714	74		106		
Totals			25.42	1347.8	65.5	2059		70		1.95	62
F04D	2.69	1330	3.50	420.4	362.4	116	74		116		
		1550	2.33	430.3	589.5	73	114		178		
		0950	18.00	1589.8	406.6	391	55		86		
Totals			23.83	2440.5	420.8	580		64		1.79	61
F08GPD	2.38	1730	10.65	320.6	81.7	334	18		60		
		0600	12.50	662.9	95.8	692	37		123		
Totals			23.15	983.5	95.9	1026		30		1.33	39
F11D	0.34	1400	5.67	184.8	72.2	256	160		113		
		2045	6.75	185.9	68.8	270	135		96		
		0815	11.50	358.7	110.0	326	153		109		
Totals			23.92	729.4	85.6	852		141		1.46	167
F12GPD	0.58	1545	7.50	128.2	46.8	274	49		58		
		0130	9.75	454.3	93.5	486	134		158		
		0815	6.75	126.6	71.9	176	54		64		
Totals			24.00	709.1	75.8	936		85		1.48	99
F14GPD	0.59	1300	5.75	191.9	37.2	516	94		119		
		2315	10.25	253.7	26.0	976	70		89		
		0715	8.00	223.0	87.8	254	79		100		
Totals			24.00	668.6	38.3	1746		79		1.49	91
F15GPD	0.62	1350	6.13	288.8	87.5	330	127		118		
		2150	8.00	353.6	92.1	384	119		110		
		0745	9.92	324.8	186.7	174	88		81		
Totals			24.05	967.2	108.9	888		108		1.55	121
F16GPD	0.72	1400	6.07	186.1	82.3	226	71		89		
		2245	8.75	306.4	40.5	756	81		101		
		0800	9.25	340.0	38.2	889	85		106		
Totals			24.07	832.5	44.5	1871		80		1.61	86
F17GPD	1.05	1200	3.93	182.0	82.0	222	74		107		
		2245	10.75	487.8	112.9	432	72		104		
		0800	9.25	366.7	92.6	396	63		91		
Totals			23.93	1036.5	98.7	1050		69		1.63	73
F18GPD	0.64	1325	5.92	150.1	61.0	246	66		89		
		2305	9.67	285.2	71.0	402	77		104		
		0730	8.41	242.9	53.5	454	75		101		
Totals			24.00	678.2	61.5	1102		74		1.49	85

Appendix E5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in DIGOXIN Study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F19GPD	1.27	1330	5.30	276.7	28.3	979	69		108		
		2240	9.17	427.0	83.4	512	61		95		
		0815	9.58	469.5	90.5	519	64		100		
Totals			24.05	1173.2	58.4	2010		64		1.87	59
F20D	0.82	1330	5.58	88.8	40.0	222	32		73		
		1930	6.00	156.9	53.7	292	53		120		
		0750	12.33	273.1	104.2	262	45		102		
Totals			23.91	518.8	66.9	776		44		1.61	47
F21GPD	1.18	1310	7.17	328.0	96.5	340	65		232		
		0630	17.33	163.9	109.2	150	13		46		
Totals			24.50	491.9	100.4	490		28		1.40	35
F22D	1.18	1200	5.50	194.0	32.2	602	50		161		
		1930	7.50	103.0	37.9	272	19		61		
		0600	10.50	224.1	81.2	276	30		97		
Totals			23.50	521.1	45.3	1150		31		1.59	34
F24GPD	0.72	1120	4.83	81.0	10.8	750	39		126		
		1630	5.17	73.2	81.3	90	33		106		
		0630	14.00	170.5	106.6	160	28		90		
Totals			24.00	324.7	32.5	1000		31		1.36	40
F29GPD	0.69	1145	4.00	47.4	37.6	126	29		83		
		1930	7.75	41.5	90.2	46	13		37		
		0800	12.50	263.4	82.8	318	51		146		
Totals			24.25	352.3	71.9	490		35		1.40	43
F30D	2.32	0700	10.00	98.5	133.1	74	7		78		
		1500	8.00	141.9	31.1	456	13		144		
		2120	6.33	77.5	26.7	290	9		100		
Totals			24.33	317.9	38.8	820		9		1.51	11
F33D	0.88	1140	3.50	38.2	15.9	240	21		78		
		2030	8.83	111.9	32.9	340	24		89		
		0900	12.50	204.6	99.3	206	31		115		
Totals			24.83	354.7	45.1	786		27		1.43	33
F34GPD	0.60	1400	6.00	124.9	24.9	502	58		94		
		1905	5.08	126.3	63.2	200	69		111		
		0800	12.92	281.2	127.8	220	60		97		
Totals			24.00	532.4	57.7	922		62		1.68	63
F37GPD	0.67	1330	5.25	164.1	15.1	1087	78		159		
		2220	8.83	261.2	76.8	340	74		151		
		0815	9.92	51.0	232.0	22	13		27		
Totals			24.00	476.3	32.9	1449		49		1.45	59
F44D	0.34	1415	6.25	184.0	167.2	110	144		113		
		2210	7.92	209.0	133.1	157	129		102		
		0920	11.17	262.0	42.7	614	115		91		
Totals			25.34	655.0	74.3	881		127		1.39	158

Appendix E5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in DIGOXIN Study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F46GPD	0.75	1600	7.50	251.2	103.8	242	74		95		
		2130	5.50	204.9	84.0	244	83		106		
		0832	11.03	390.8	46.0	850	79		101		
Totals			24.03	846.9	63.4	1336		78		1.56	87
F52GPD	1.61	1415	8.75	424.9	107.8	394	50		78		
		2100	6.75	377.9	57.4	658	58		91		
		0530	8.50	680.2	97.2	700	83		130		
Totals			24.00	1482.0	84.6	1752		64		1.70	65
F54GPD	1.66	1245	4.42	281.4	44.1	638	64		123		
		2200	9.25	397.6	126.6	314	43		83		
		0820	10.33	461.5	169.7	272	45		87		
Totals			24.00	1140.5	101.5	1224		52		1.70	53
F01D	0.62									1.17	
F05D	2.19									1.44	
M03D	2.08	0830	10.50	1080.5	114.3	945	82		105		
		1845	10.25	1198.0	120.4	995	94		121		
		0100	6.25	345.6	104.7	330	44		56		
Totals			27.00	2624.1	115.6	2270		78		1.93	70
M06D	2.37	1530	6.17	479.7	58.1	825	55		120		
		1940	4.17	102.6	131.5	78	17		37		
		0920	13.67	974.9	212.4	459	50		109		
Totals			24.01	1557.2	114.3	1362		46		1.71	46
M09GPD	1.18	1330	6.50	654.9	153.0	428	142		134		
		2200	8.50	785.2	133.0	590	130		123		
		0730	9.50	398.2	110.6	360	59		56		
Totals			24.50	1838.3	133.4	1378		106		2.20	83
M10GPD	1.16	1630	7.12	183.7	113.4	162	37		82		
		2330	7.00	180.8	83.7	216	37		82		
		0925	10.92	412.6	86.7	476	54		120		
Totals			25.04	777.1	91.0	854		45		1.79	43
M13GPD	1.05	1400	5.00	407.1	46.5	876	129		155		
		2115	7.25	462.3	122.3	378	101		122		
		0900	11.75	388.2	183.1	212	52		63		
Totals			24.00	1257.6	85.8	1466		83		1.91	75
M23GPD	0.55	0900	10.75	664.7	117.0	568	187		97		
		1530	6.50	449.6	23.8	1888	209		108		
		2215	6.75	415.3	64.0	649	186		96		
Totals			24.00	1529.6	49.3	3105		193		2.04	164
M25D	0.73	1500	4.00	74.0	127.6	58	42		145		
		1915	4.25	27.9	139.6	20	15		52		
		1100	15.75	200.5	37.4	536	29		100		
Totals			24.00	302.4	49.3	614		29		1.36	37

Appendix E5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in DIGOXIN Study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M26D	0.98	1445	6.25	717.2	99.3	722	195		181		
		2130	6.75	439.8	81.4	540	111		103		
		0945	12.25	455.6	159.3	286	63		58		
Totals			25.25	1609.6	104.0	1548		108		2.00	94
M31GPD	0.80	1135	3.92	202.5	151.1	134	108		177		
		1730	5.91	140.2	143.1	98	49		80		
		0710	13.67	345.0	132.7	260	53		87		
Totals			23.50	687.7	139.8	492		61		1.80	59
M35GPD	0.91	1345	5.42	289.3	272.9	106	98		115		
		2230	8.75	460.0	86.5	532	96		113		
		0815	9.75	358.6	57.3	626	67		79		
Totals			23.92	1107.9	87.7	1264		85		1.69	87
M36GPD	1.45	1550	7.83	555.8	402.7	138	82		94		
		2225	6.58	507.3	248.7	204	89		102		
		0717	8.87	703.0	155.5	452	91		104		
Totals			23.28	1766.1	222.4	794		87		2.00	75
M38GPD	1.10	1250	5.08	287.2	105.6	272	86		105		
		2200	9.17	496.3	143.4	346	82		100		
		0820	10.33	553.7	86.5	640	81		99		
Totals			24.58	1337.2	106.3	1258		82		1.68	85
M40GPD	0.92	1505	7.08	372.9	126.9	294	95		95		
		2100	5.92	358.4	122.7	292	110		110		
		0750	10.83	577.3	106.5	542	97		97		
Totals			23.83	1308.6	116.0	1128		100		1.98	87
M41GPD	0.61	1545	7.75	271.3	222.3	122	96		91		
		2100	5.25	235.8	94.3	250	123		117		
		0800	11.00	415.8	46.5	894	103		98		
Totals			24.00	922.9	72.9	1266		105		1.88	97
M42GPD	1.41	1345	4.80	432.3	184.7	234	106		156		
		2252	9.12	427.9	105.9	404	55		81		
		0906	10.23	530.4	73.1	726	61		90		
Totals			24.15	1390.6	101.9	1364		68		1.82	65
M43GPD	1.09	1357	5.98	387.5	60.4	642	99		113		
		2242	8.75	549.0	68.3	804	96		109		
		0801	9.32	453.1	49.8	910	74		84		
Totals			24.05	1389.6	59.0	2356		88		1.91	80
M47GPD	1.41	1515	7.25	334.8	37.0	904	55		117		
		2115	6.00	229.9	74.7	308	45		96		
		0813	10.97	391.6	108.2	362	42		89		
Totals			24.22	956.3	60.8	1574		47		1.92	42
M48GPD	1.28	1510	9.33	395.5	138.3	286	55		98		
		2320	8.17	462.0	70.0	660	74		132		
		0540	6.33	169.2	30.4	556	35		63		
Totals			23.83	1026.7	68.4	1502		56		1.85	53

Appendix E5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in DIGOXIN Study (contd)

subject	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M49D	0.95	1430	6.00	185.7	24.6	755	54		113		
		2205	7.58	166.4	58.6	284	39		81		
		0830	10.42	304.5	45.6	668	51		106		
Totals			24.00	656.6		1707		48		1.84	45
M50GPD	0.91	1515	7.08	22.5	31.1	1679	135		155		
		2130	6.25	350.7	83.9	418	103		118		
		0825	10.92	775.0	47.1	1645	130		149		
Totals			24.25	1148.2	30.7	3742		87		1.90	79
M51GPD	1.16	1400	6.25	372.9	181.0	206	86		84		
		2330	9.50	747.1	125.8	594	113		111		
		0745	8.25	589.6	70.7	834	103		101		
Totals			24.00	1709.6	104.6	1634		102		2.18	81
M53GPD	1.13	0757	10.87	707.2	106.2	666	96		116		
		1456	6.98	332.4	63.4	524	70		84		
		2022	5.43	268.4	124.3	216	73		88		
Totals			23.28	1308.0	93.0	1406		83		1.77	81
M55GPD	0.77	1525	7.67	326.9	21.1	1550	92		96		
		2230	7.08	423.8	56.9	745	130		135		
		0745	9.25	313.0	62.6	500	73		76		
Totals			24.00	1063.7	38.1	2795		96		1.62	103

Appendix E6 Summary of Results From All Female Subjects Taking DIGOXIN

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	26	73	96	77	84	81.5	81.9	5.8
mobility score	26	1	5	2	4	3.0	2.8	1.4
urine volume (ml)	24	490	2059	828	1421	1013	1133	461
UCr (mg in 24h)	24	318	2441	499	1024	693.7	816.0	474.5
[UCr] (mg/100ml)	24	32.5	420.8	45.1	93.3	66.2	82.1	75.8
[SCr] (mg/100ml)	26	0.34	2.69	0.62	1.36	0.785	1.092	0.662
CCr (ml/min)	24	9	141	32	79	63.7	62.5	32.0
CCr/SA (ml/min/1.73m2)	24	11	167	41	87	61.6	69.7	37.9
dose (ug)	26	62.5	250.0	62.5	156.2	125.0	134.6	70.0
%Du	24	19.0	82.8	32.1	62.4	38.7	45.28	18.53
UDIG (ug in 24h)	24	19.4	165.9	31.4	77.1	51.24	56.95	32.7
[UDIG] (ug/100ml)	24	1.5	18.8	2.9	7.1	4.7	5.79	4.27
[SDIG] (ng/ml)	26	0.14	3.00	0.382	1.26	0.740	0.913	0.715
Clt (ml/min)	26	19	224	54	123	87.3	94.9	53.6
Clt/SA (ml/min/1.73m2)	26	23	265	60	146	98.3	107.9	62.0
Clt/kg (ml/min/kg)	26	0.398	4.474	1.011	2.430	1.627	1.804	1.039
Clr (ml/min)	24	10	171	34	90	51.1	66.3	46.7
Clr/SA (ml/min/1.73m2)	24	11	199	34	109	58.7	74.3	53.1
Clr/kg (ml/min/kg)	24	0.184	3.298	0.547	1.990	0.976	1.234	0.882
Clr/CCr	24	0.326	2.461	0.689	1.446	0.970	1.091	0.565
Clnr (ml/min)	24	-30	105	5	61	40.3	34.5	33.9
Clnr/kg (ml/min/kg)	24	-0.583	2.092	0.109	1.069	0.589	0.637	0.647
weight (kg)	26	35	76	46	60	52.5	53.9	10.1
SA (m2)	26	1.17	1.95	1.42	1.64	1.50	1.54	0.17

Appendix E7 Spearmans Coefficient of Rank Correlation for All Female Subjects Taking DIGOXIN

	age years	M.Sc	weight kg	SCr mg/dl	CCr ml/min	dose mcg	%Du	UDIG mcg	SDIG ng/ml	Clt ml/min	Clt/kg ml/min /kg	Clr ml/min	Clr/kg ml/min /kg	Clnr ml/min
n	26	26	26	26	24	26	24	24	26	26	26	24	24	24
M.Sc	0.213													
weight	-0.068	-0.248												
SCr	0.172	0.323	0.353											
CCr	-0.375	-0.527	0.162	-0.612 ***										
dose	0.066	0.017	0.003	-0.206	0.068									
%Du	-0.346	-0.168	-0.090	-0.426	0.395	-0.346								
UDIG	-0.124	-0.053	0.018	-0.413	0.219	0.723 ****	0.365							
SDIG	0.406	0.414	-0.075	0.285	-0.477	0.585 ***	-0.334	0.440						
Clt	-0.426	-0.542 ***	0.107	-0.515 ***	0.659 ***	0.055	0.086	0.026	-0.744 ****					
Clt/kg	-0.504	-0.527 ***	-0.100	-0.584 ***	0.667 ***	0.041	0.152	0.035	-0.732 ****	0.960 ****				
Clr	-0.527	-0.493	0.002	-0.631 ***	0.732 ****	-0.080	0.633 ***	0.280	-0.669 ****	0.799 ****	0.816 ****			
Clr/kg	-0.483	-0.349	-0.247	-0.668 ***	0.611 ***	-0.173	0.642 ***	0.200	-0.712 ****	0.747 ****	0.819 ****	0.936 ****		
Clnr	-0.070	-0.126	0.084	0.020	0.154	0.229	-0.611 ***	-0.277	-0.245	0.538 ***	0.474	0.035	0.014	
Clnr/kg	0.014	-0.086	-0.034	-0.007	0.095	0.229	-0.658 ***	-0.310	-0.194	0.469	0.425	-0.042	-0.028	0.980 ****

*** p<0.01 **** p<0.001

Appendix E8 Summary of Results From All Male Subjects Taking DIGOXIN

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	23	66	89	75	81	79.0	77.8	6.4
mobility score	23	1	4	1	3	2.0	2.2	1.2
urine volume (ml)	23	492	3742	1258	1707	1406	1603	784
UCr (mg in 24h)	23	302	2624	956	1557	1308	1273	484
[UCr] (mg/100ml)	23	30.7	222.4	59.0	114.3	91.00	90.56	42.3
[SCr] (mg/100ml)	23	0.55	2.37	0.91	1.28	1.090	1.130	0.423
CCr (ml/min)	23	29	193	56	99	83.1	81.9	33.4
CCr/SA (ml/min/1.73m2)	23	37	164	52	87	79.0	75.2	27.2
dose (ug)	23	62.5	250.0	125.0	250.0	125.0	152.2	70.1
%Du	23	25.0	84.9	36.0	53.7	46.7	47.73	16.35
UDIG (ug in 24h)	23	22.8	163.9	47.0	88.9	62.00	69.24	34.33
[UDIG] (ug/100ml)	23	1.2	12.7	2.7	6.9	4.2	5.08	3.05
[SDIG] (ng/ml)	23	0.26	1.70	0.41	0.76	0.520	0.654	0.357
Clt (ml/min)	23	34	264	77	164	111.8	127.8	69.2
Clt/SA (ml/min/1.73m2)	23	35	229	76	158	107.5	117.4	59.5
Clt/kg (ml/min/kg)	23	0.552	3.462	1.173	2.560	1.780	1.583	0.900
Clr (ml/min)	23	27	194	53	116	81.2	85.0	45.1
Clr/SA (ml/min/1.73m2)	23	28	168	51	97	71.0	77.7	38.0
Clr/kg (ml/min/kg)	23	0.422	2.281	0.787	1.449	1.041	1.167	0.545
Clr/CCr	23	0.546	1.788	0.699	1.228	1.034	1.060	0.337
Clnr (ml/min)	23	-35	130	7	79	30.6	39.7	44.1
Clnr/kg (ml/min/kg)	23	-0.416	1.734	0.111	0.848	0.411	0.581	0.629
weight (kg)	23	41	96	64	78	74.0	71.6	12.4
SA (m2)	23	1.36	2.20	1.77	1.98	1.880	1.860	0.182

Appendix E9 Spearmans Coefficient of Rank Correlation for All Male Subjects Taking DIGOXIN

	age years	M.Sc	weight kg	SCr mg/dl	CCr ml/min	dose mcg	%Du	UDIG mcg	SDIG ng/ml	Clt ml/min	Clt/kg ml/min /kg	Clr ml/min	Clr/kg ml/min /kg	Clnr ml/min
n	23	23	23	23	23	23	23	23	23	23	23	23	23	23
M.Sc	0.236													
weight	-0.447	-0.350												
SCr	-0.164	0.041	0.226											
CCr	-0.504	-0.570 ***	0.584 ***	-0.340										
dose	-0.606 ***	-0.405	0.357	-0.067	0.498									
%Du	-0.007	0.131	0.253	0.173	0.145	-0.290								
UDIG	-0.594 ***	-0.292	0.605 ***	0.047	0.588 ***	0.759 ****	0.366							
SDIG	-0.081	0.021	0.016	0.141	-0.182	0.317	0.166	0.418						
Clt	-0.453	-0.377	0.300	-0.171	0.573 ***	0.626 ***	-0.401	0.324	-0.536					
Clt/kg	-0.329	-0.326	-0.010	-0.299	0.456	0.495	-0.463	0.131	-0.609 ***	0.939 ****				
Clr	-0.403	-0.378	0.526	-0.122	0.731 ****	0.462	0.179	0.555 ***	-0.474	0.799 ****	0.691 ***			
Clr/kg	-0.377	-0.269	0.270	-0.159	0.623 ***	0.348	0.156	0.401	-0.589 ***	0.788 ****	0.776 ****	0.931 ****		
Clnr	-0.112	-0.286	-0.126	-0.229	0.088	0.431	-0.933 ****	-0.193	-0.300	0.637 ***	0.678 ***	0.101	0.110	
Clnr/kg	-0.046	-0.264	-0.272	-0.301	0.057	0.324	-0.923 ****	-0.299	-0.356	0.583 ***	0.686 ***	0.045	0.106	0.977 ****

*** p<0.01 **** p<0.001

Appendix E10 Female v Male Elderly Taking DIGOXIN - Mann-Whitney Test

	female n	male n	female median	male median	female mean	male mean	female s.d.	male s.d.	p
age (years)	26	23	81.5	79.0	81.9	77.8	5.8	6.4	
mobility score	26	23	3.0	2.0	2.8	2.2	1.4	1.2	
urine volume (ml)	24	23	1013	1406	1133	1603	461	784	**
UCr (mg in 24h)	24	23	694	1308	816	1273	475	484	***
[UCr] (mg/100ml)	24	23	66.2	91.0	82.1	90.6	75.8	42.3	
[SCr] (mg/100ml)	26	23	0.785	1.090	1.092	1.130	0.662	0.423	
CCr (ml/min)	24	23	63.7	83.2	62.5	81.9	32.0	33.4	*
CCr/SA (ml/min/1.73m2)	24	23	61.6	79.0	69.7	75.2	37.9	27.2	
dose (mcg)	26	23	125.0	125.0	134.6	152.2	70.0	70.1	
%Du	24	23	38.67	46.72	45.28	47.73	18.53	16.35	
UDIG (mcg in 24h)	24	23	51.24	62.00	56.95	69.24	32.70	34.33	
[UDIG] (mcg/100ml)	24	23	4.742	4.179	5.791	5.078	4.266	3.052	
[SDIG] (ng/ml)	26	23	0.740	0.520	0.913	0.654	0.715	0.357	
Clt (ml/min)	26	23	87.3	111.8	94.9	127.8	53.6	69.2	
Clt/SA (ml/min/1.73m2)	26	23	98.3	107.5	107.9	117.4	62.0	59.5	
Clt/kg (ml/min/kg)	26	23	1.627	1.583	1.804	1.780	1.039	0.900	
Clr (ml/min)	24	23	51.1	81.2	66.3	85.0	46.7	45.1	
Clr/SA (ml/min/1.73m2)	24	23	58.7	71.0	74.3	77.7	53.1	38.1	
Clr/kg (ml/min/kg)	24	23	0.976	1.041	1.234	1.167	0.882	0.545	
Clnr (ml/min)	24	23	40.3	30.6	34.5	39.7	33.9	44.1	
Clnr/kg (ml/min/kg)	24	23	0.589	0.411	0.637	0.581	647	0.629	
Clr/CCr	24	23	0.970	1.060	1.091	1.034	0.565	0.337	
weight (kg)	26	23	52.5	74.0	53.9	71.6	10.1	12.4	***
SA (m2)	26	23	1.500	1.880	1.540	1.860	0.174	0.182	***

* p<0.03 ** p<0.02 *** p<0.001

Appendix E11 Summary of Results From All Subjects Taking DIGOXIN

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	49	66	96	77	84	80.0	80.0	6.3
mobility score	49	1	5	1	4	3.0	2.5	1.3
urine volume (ml)	47	490	3742	881	1634	1264	1363	676
UCr (mg in 24h)	47	302	2624	657	1348	983.5	1039.6	527.2
[UCr] (mg/100ml)	47	30.7	420.8	49.3	101.9	74.30	86.21	61.21
[SCr] (mg/100ml)	49	0.34	2.69	0.71	1.28	0.980	1.110	0.557
CCr (ml/min)	47	9	193	47	87	69.6	72.0	33.8
CCr/SA (ml/min/1.73m2)	47	11	167	46	87	69.8	72.4	32.9
dose (ug)	49	62.5	250.0	93.8	250.0	125.0	142.9	69.9
%Du	47	19.04	84.88	33.44	62.37	42.31	46.48	17.35
UDIG (ug in 24h)	47	19.4	165.9	41.3	78.0	54.00	62.97	33.71
[UDIG] (ug/100ml)	47	1.17	18.83	2.83	6.85	4.37	5.44	3.70
[SDIG] (ng/ml)	49	0.14	3.00	0.40	1.02	0.590	0.792	0.585
Clt (ml/min)	49	19	264	59	144	98.6	110.3	63.0
Clt/SA (ml/min/1.73m2)	49	23	265	65	154	99.6	112.4	60.4
Clt/kg (ml/min/kg)	49	0.398	4.474	1.036	2.468	1.583	1.793	0.966
Clr (ml/min)	47	10	194	41	109	60.4	75.4	46.4
Clr/SA (ml/min/1.73m2)	47	11	199	41	97	59.3	76.0	45.8
Clr/kg (ml/min/kg)	47	0.184	3.298	0.717	1.469	1.004	1.201	0.729
Clr/CCr	47	0.326	2.461	0.699	1.314	1.007	1.063	0.463
Clnr (ml/min)	47	-35	130	6.87	64	31.5	37.0	38.9
Clnr/kg (ml/min/kg)	47	-0.583	2.092	0.111	1.015	0.508	0.610	0.632
weight (kg)	49	35	96	51	75	61.0	62.2	14.2
SA (m2)	49	1.17	2.20	1.49	1.89	1.690	1.690	0.239

Appendix E12 Spearmans Coefficient of Rank Correlation for All Subjects Taking DIGOXIN

	age years	M.Sc	weight kg	SCr mg/dl	CCr ml/min	dose mcg	%Du	UDIG mcg	SDIG ng/ml	Clt ml/min	Clt/kg ml/min /kg	Clr ml/min	Clr/kg ml/min /kg	Clnr ml/min
n	49	49	49	49	47	49	47	47	49	49	49	47	47	47
M.Sc	0.282													
weight	-0.369	-0.372 ***												
SCr	0.027	0.212	0.356											
CCr	-0.507 ****	-0.507 ****	0.424 ***	-0.378										
dose	-0.255	-0.203	0.234	-0.160	0.300									
%Du	-0.207	-0.041	0.174	-0.153	0.274	0.303								
UDIG	-0.388 ***	-0.195	0.394 ***	-0.196	0.094	0.742 ****	0.380 ***							
SDIG	0.231	0.286	0.066	0.202	-0.371	0.454 ***	-0.109	0.407 ***						
Clt	-0.487 ****	-0.492 ****	0.264	-0.345	0.653 ****	0.318	-0.132	0.189	-0.669 ****					
Clt/kg	-0.390 ***	-0.408 ***	-0.092	-0.486 ****	0.523 ****	0.204	-0.186	0.021	-0.694 ****	0.920 ****				
Clr	-0.527 ****	-0.429 ***	0.314	-0.375	0.754 ****	0.142	0.417 ***	0.384 ***	-0.633 ****	0.826 ****	0.729 ****			
Clr/kg	-0.466 ***	-0.333	-0.009	-0.478 ***	0.642 ****	0.043	0.399 ***	0.267	-0.673 ****	0.775 ****	0.796 ****	0.926 ****		
Clnr	-0.065	-0.212	-0.038	-0.074	0.116	0.360	-0.815 ****	-0.216	-0.233	0.552 ****	0.591 ****	0.049	0.054	
Clnr/kg	0.032	-0.134	-0.194	-0.114	0.037	0.289	-0.838 ***	-0.305	-0.221	0.471 ***	0.560 ****	-0.038	0.018	0.977 ****

*** p<0.01 **** p<0.001

Appendix E13 Fit v Frail Elderly Subjects Taking DIGOXIN - Mann-Whitney Test

	fit n	frail n	fit min	frail min	fit max	frail max	fit Q1	frail Q1	fit Q3	frail Q3
age (years)	24	25	66	67	90	96	76	78	84	85
mobility score	24	25	1	3	2	5	1	3	2	4
urine volume (ml)	23	24	794	490	3105	3742	1224	778	1752	1513
UCr (mg in 24h)	23	24	476	302	1838	2624	850	499	1391	1127
[UCr] (mg/100ml)	23	24	32.9	30.7	222.4	420.8	59.0	45.1	104.6	100.0
[SCr] (mg/100ml)	24	25	0.55	0.34	1.66	2.69	0.63	0.75	1.27	1.75
CCr (ml/min)	23	24	49	9	193	141	68	31	99	82
CCr/SA (ml/min/1.73m2)	23	24	52	11	164	167	65	39	87	77
dose (ug)	24	25	62.5	62.5	250.0	250.0	78.1	93.7	250.0	125.0
%Du	23	24	25.3	19.0	82.8	84.9	34.0	31.5	50.4	64.8
UDIG (ug in 24h)	23	24	20.9	19.4	125.9	165.9	43.4	27.4	85.1	78.0
[UDIG] (ug/100ml)	23	24	1.53	1.17	9.14	18.83	2.83	2.57	4.86	9.38
[SDIG] (ng/ml)	24	25	0.14	0.26	1.85	3.00	0.38	0.40	0.81	1.28
Clt (ml/min)	24	25	35	19	264	224	83	52	195	118
Clt/SA (ml/min/1.73m2)	24	25	31	23	229	265	83	57	175	122
Clt/kg (ml/min/kg)	24	25	0.461	0.398	3.462	4.474	1.238	0.984	3.025	1.899
Clr (ml/min)	23	24	31	10	182	194	50	30	117	85
Clr/SA (ml/min/1.73m2)	23	24	29	11	199	180	53	34	99	89
Clr/kg (ml/min/kg)	23	24	0.422	0.184	3.298	2.820	0.808	0.583	1.479	1.348
Clnr (ml/min)	23	24	-30	-35	130	105	13	3	70	44
Clnr/kg (ml/min/kg)	23	24	-0.583	-0.416	1.923	2.092	0.194	0.049	1.183	0.905
Clr/CCr	23	24	0.500	0.326	2.179	2.461	0.666	0.707	1.139	1.400
weight (kg)	24	25	35	41	96	85	54	46	76	66
SA (m2)	24	25	1.17	1.33	2.20	2.00	1.55	1.42	1.94	1.80

Appendix E13 Fit v Frail Elderly Subjects Taking DIGOXIN - Mann-Whitney Test (contd)

	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	79.0	81.0	78.9	81.0	5.9	6.7	
mobility score	1.0	3.0	1.3	3.6	0.5	0.8	****
urine volume (ml)	1378	961	1517	1216	520	780	**
UCr (mg in 24h)	1173	709	1170	915	371	625	**
[UCr] (mg/100ml)	75.8	73.1	84.5	87.9	40.0	77.2	
[SCr] (mg/100ml)	0.985	0.980	0.988	1.228	0.353	0.688	
CCr (ml/min)	82.4	47.4	85.2	59.4	29.0	33.7	***
CCr/SA (ml/min/1.73m2)	83.3	46.8	82.6	62.6	24.1	37.4	***
dose (ug)	125.0	125.0	151.0	135.0	75.8	64.2	
%Du	42.32	40.76	46.06	46.88	6.00	18.89	
UDIG (ug in 24h)	58.40	53.00	63.30	62.65	26.95	39.73	
[UDIG] (ug/100ml)	4.18	5.06	4.41	6.43	1.95	4.65	
[SDIG] (ng/ml)	0.500	0.720	0.628	0.949	0.393	0.695	
Clt (ml/min)	117.5	74.6	132.8	88.7	66.7	51.8	**
Clt/SA (ml/min/1.73m2)	122.4	75.9	131.7	93.9	60.6	55.2	*
Clt/kg (ml/min/kg)	2.098	1.206	2.060	1.537	0.968	0.911	*
Clr (ml/min)	81.2	53.9	86.1	65.2	43.4	47.8	
Clr/SA (ml/min/1.73m2)	79.6	55.3	84.4	67.9	43.9	47.1	
Clr/kg (ml/min/kg)	1.187	0.927	1.309	1.098	0.722	0.736	
Clnr (ml/min)	41.2	24.9	45.5	28.9	41.7	35.0	
Clnr/kg (ml/min/kg)	0.553	0.927	0.684	1.098	0.649	0.736	
Clr/CCr	0.981	1.062	1.006	1.117	0.423	0.502	
weight (kg)	67.5	59.0	66.5	58.1	14.7	12.7	*
SA (m2)	1.760	1.620	1.757	1.626	0.253	0.201	*

* p<0.05 ** p<0.02 *** p<0.003 **** p<0.001

Appendix E14 Fit v Frail Elderly Females Taking DIGOXIN - Mann-Whitney Test

	fit n	frail n	fit min	frail min	fit max	frail max	fit Q1	frail Q1	fit Q3	frail Q3
age (years)	12	14	73	74	90	96	77	77	84	90
mobility score	12	14	1	3	2	5	1	3	2	5
urine volume (ml)	11	13	888	490	2059	1150	1102	678	1871	1013
UCr (mg in 24h)	11	13	476	318	1482	2441	678	354	1173	856
[UCr] (mg/100ml)	11	13	32.9	32.5	108.9	420.8	44.5	45.2	84.6	97.3
[SCr] (mg/100ml)	12	14	0.58	0.34	1.66	2.69	0.62	0.67	1.27	2.22
CCr (ml/min)	11	13	49	9	108	141	64	29	80	66
CCr/SA (ml/min/1.73m2)	11	13	53	11	121	167	59	35	91	68
dose (ug)	12	14	62.5	62.5	250.0	250.0	62.5	109.4	218.7	156.2
%Du	11	13	25.3	19.0	82.8	69.5	33.4	30.4	68.9	62.4
UDIG (ug in 24h)	11	13	20.9	19.4	97.7	165.9	39.8	23.8	63.3	78.0
[UDIG] (ug/100ml)	11	13	1.53	2.33	5.96	18.83	2.51	3.85	4.86	9.51
[SDIG] (ng/ml)	12	14	0.14	0.26	1.85	3.00	0.30	0.47	0.87	1.47
Clt (ml/min)	12	14	35	19	208	224	87	44	131	99
Clt/SA (ml/min/1.73m2)	12	14	31	23	223	265	87	54	166	119
Clt/kg (ml/min/kg)	12	14	0.461	0.398	3.462	4.474	1.277	0.961	2.745	2.024
Clr (ml/min)	11	13	32	10	172	169	43	23	107	77
Clr/SA (ml/min/1.73m2)	11	13	33	11	199	180	41	25	124	89
Clr/kg (ml/min/kg)	11	13	0.515	0.184	3.298	2.820	0.611	0.408	2.224	1.602
Clnr (ml/min)	11	13	-30	-2	98	105	-3	6	68	46
Clnr/kg (ml/min/kg)	11	13	-0.583	-0.039	1.923	2.092	-0.070	0.137	1.183	0.971
Clr/CCr	11	13	0.500	0.326	2.179	2.461	0.666	0.704	1.452	1.621
weight (kg)	12	14	35	41	76	66	51	45	65	59
SA (m2)	12	14	1.17	1.33	1.95	1.79	1.48	1.40	1.70	1.62

Appendix E14 Fit v Frail Elderly Females Taking DIGOXIN - Mann-Whitney Test (contd)

	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	80.5	82.0	80.7	83.0	4.6	6.6	
mobility score	1.5	4.0	1.5	3.9	0.5	0.9	***
urine volume (ml)	1449	852	1488	833	423	210	***
UCr (mg in 24h)	850	521	939	712	314	569	*
[UCr] (mg/100ml)	63.4	71.9	66.9	94.9	24.4	100.6	
[SCr] (mg/100ml)	0.695	0.965	0.917	1.243	0.414	0.803	
CCr (ml/min)	73.5	35.1	73.0	53.7	16.3	39.5	**
CCr/SA (ml/min/1.73m2)	85.4	43.4	78.9	61.9	21.0	47.4	*
dose (ug)	125.0	125.0	130.2	138.4	77.5	65.7	
%Du	39.10	38.24	47.03	43.79	20.19	17.70	
UDIG (ug in 24h)	49.4	52.0	52.4	60.8	22.7	39.8	
[UDIG] (ug/100ml)	3.91	5.95	3.65	7.60	1.43	5.04	*
[SDIG] (ng/ml)	0.490	1.015	0.632	1.154	0.476	0.811	
Clt (ml/min)	113.0	62.5	112.4	79.9	43.1	58.6	*
Clt/SA (ml/min/1.73m2)	131.4	69.3	127.0	91.6	52.6	66.6	
Clt/kg (ml/min/kg)	2.253	1.099	2.102	1.548	0.919	1.100	
Clr (ml/min)	62.8	42.6	76.7	57.5	45.1	48.1	
Clr/SA (ml/min/1.73m2)	59.6	55.4	84.2	66.0	53.1	53.7	
Clr/kg (ml/min/kg)	0.980	0.972	1.371	1.118	0.896	0.889	
Clnr (ml/min)	41.5	22.9	38.7	30.9	38.6	30.4	
Clnr/kg (ml/min/kg)	0.624	0.509	0.683	0.598	0.732	0.593	
Clr/CCr	0.776	0.980	1.040	1.134	0.536	0.607	
weight (kg)	54.0	48.5	57.0	51.3	11.6	8.1	
SA (m2)	1.555	1.450	1.585	1.501	0.205	0.137	

* $p<0.04$ ** $p<0.02$ *** $p<0.001$

Appendix E15 Fit v Frail Elderly Males Taking DIGOXIN - Mann-Whitney Test

	fit n	frail n	fit min	frail min	fit max	frail max	fit Q1	frail Q1	fit Q3	frail Q3
age (years)	12	11	66	67	89	87	71	79	82	81
mobility score	12	11	1	3	2	4	1	3	1	4
urine volume (ml)	12	11	794	492	3105	3742	1260	854	1601	2270
UCr (mg in 24h)	12	11	923	302	1838	2624	1145	688	1665	1557
[UCr] (mg/100ml)	12	11	49.3	30.7	222.4	139.8	69.5	38.5	113.6	114.3
[SCr] (mg/100ml)	12	11	0.55	0.73	1.45	2.37	0.91	0.80	1.26	1.41
CCr (ml/min)	12	11	56	29	193	108	83	46	104	87
CCr/SA (ml/min/1.73m2)	12	11	52	37	164	103	75	43	87	81
dose (ug)	12	11	62.5	62.5	250.0	250.0	125.0	62.5	250.0	125.0
%Du	12	11	25.6	25.0	71.1	84.9	37.5	35.8	50.2	69.1
UDIG (ug in 24h)	12	11	32.7	22.8	125.9	163.9	54.3	32.6	89.7	86.4
[UDIG] (ug/100ml)	12	11	2.60	1.17	9.14	12.70	3.82	2.07	7.41	6.85
[SDIG] (ng/ml)	12	11	0.26	0.30	1.30	1.70	0.44	0.38	0.76	0.98
Clt (ml/min)	12	11	45	34	264	164	80	59	230	142
Clt/SA (ml/min/1.73m2)	12	11	42	35	229	158	71	76	212	132
Clt/kg (ml/min/kg)	12	11	0.605	0.552	3.462	2.579	1.056	1.173	3.199	1.826
Clr (ml/min)	12	11	31	27	182	194	63	52	119	109
Clr/SA (ml/min/1.73m2)	12	11	29	28	154	168	57	49	98	97
Clr/kg (ml/min/kg)	12	11	0.422	0.442	2.272	2.281	0.867	0.777	1.460	1.449
Clnr (ml/min)	12	11	-4	-35	130	99	16	-2	81	44
Clnr/kg (ml/min/kg)	12	11	-0.053	-0.416	1.734	1.682	0.226	-0.022	1.197	0.839
Clr/CCr	12	11	0.546	0.575	1.540	1.788	0.643	0.699	1.138	1.396
weight (kg)	12	11	60	41	96	85	70	62	80	77
SA (m2)	12	11	1.68	1.36	2.20	2.00	1.83	1.71	2.03	1.92

Appendix E15 Fit v Frail Elderly Males Taking DIGOXIN - Mann-Whitney Test (contd)

	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	78.0	79.0	77.2	78.5	6.8	6.2	
mobility score	1.0	3.0	1.1	3.4	0.3	0.5	***
urine volume (ml)	1543	1669	1371	1548	614	963	
UCr (mg in 24h)	1363	1064	1382	1154	289	627	
[UCr] (mg/100ml)	94.8	91.0	100.6	79.6	45.5	37.6	
[SCr] (mg/100ml)	1.095	0.980	1.059	1.208	0.279	0.544	
CCr (ml/min)	87.8	61.0	96.4	66.1	33.9	25.6	**
CCr/SA (ml/min/1.73m2)	82.3	58.6	86.0	63.44	27.1	23.0	*
dose (ug)	125.0	125.0	171.9	130.7	71.1	65.3	
%Du	46.92	43.20	45.16	50.53	11.83	20.44	
UDIG (ug in 24h)	63.1	54.0	73.3	64.8	27.5	41.4	
[UDIG] (ug/100ml)	4.20	3.71	5.12	5.04	2.15	3.93	
[SDIG] (ng/ml)	0.510	0.590	0.623	0.689	0.310	0.414	
Clt (ml/min)	136.9	96.9	153.2	100.0	80.9	41.3	
Clt/SA (ml/min/1.73m2)	119.1	86.9	136.4	96.7	69.8	39.0	
Clt/kg (ml/min/kg)	1.733	1.447	2.017	1.522	1.054	0.645	
Clr (ml/min)	88.4	57.3	94.8	74.3	41.7	48.1	
Clr/SA (ml/min/1.73m2)	85.3	55.2	84.6	70.2	35.9	40.5	
Clr/kg (ml/min/kg)	1.207	0.822	1.253	1.074	0.554	0.545	
Clnr (ml/min)	30.7	27.0	51.8	26.5	45.0	41.2	
Clnr/kg (ml/min/kg)	0.424	0.403	0.685	0.467	0.596	0.673	
Clr/CCr	1.032	1.122	0.975	1.098	0.307	0.370	
weight (kg)	75.0	65.0	76.0	66.8	1.1	12.3	
SA (m2)	1.910	1.800	1.928	1.786	0.164	0.178	

* $p<0.05$ ** $p<0.025$ *** $p<0.001$

Appendix E16 Fit v Frail Elderly Males Matched for CCr - Mann-Whitney Test

	fit n	frail n	fit min	frail min	fit max	frail max	fit Q1	frail Q1	fit Q3	frail Q3
age (years)	11	8	66	67	89	81	75	70	82	81
mobility score	11	8	1	3	2	4	1	3	1	4
urine volume (ml)	11	8	794	492	2356	3742	1258	1373	1502	2664
UCr (mg in 24h)	11	8	923	657	1838	2624	1108	782	1710	1596
[UCr] (mg/100ml)	11	8	59.0	30.7	222.4	139.8	72.9	38.2	116.0	115.3
[SCr] (mg/100ml)	11	8	0.61	0.77	1.45	2.37	0.92	0.83	1.28	1.84
CCr (ml/min)	11	8	56	46	106	108	82	51	102	94
CCr/SA (ml/min/1.73m2)	11	8	52	45	97	103	75	49	87	91
dose (ug)	11	8	62.5	62.5	250.0	250.0	125.0	125.0	250.0	218.7
%Du	11	8	25.6	25.0	71.1	84.9	36.0	30.4	50.4	73.6
UDIG (ug in 24h)	11	8	32.7	32.6	125.9	163.9	52.9	48.7	88.9	101.2
[UDIG] (ug/100ml)	11	8	2.60	1.17	9.14	12.70	3.89	2.13	7.71	10.46
[SDIG] (ng/ml)	11	8	0.26	0.30	1.30	1.70	0.44	0.39	0.76	0.99
Clt (ml/min)	11	8	45	34	264	164	77	64	224	150
Clt/SA (ml/min/1.73m2)	11	8	42	35	229	158	67	59	211	147
Clt/kg (ml/min/kg)	11	8	0.61	0.55	3.46	2.58	0.98	0.87	3.10	2.38
Clr (ml/min)	11	8	31	27	131	194	57	53	117	114
Clr/SA (ml/min/1.73m2)	11	8	29	28	134	168	53	51	95	110
Clr/kg (ml/min/kg)	11	8	0.42	0.44	2.18	2.28	0.81	0.78	1.43	1.70
Clnr (ml/min)	11	8	-4	-35	130	99	13	-8	81	76
Clnr/kg (ml/min/kg)	11	8	-0.05	-0.42	1.73	1.68	0.19	-0.11	1.31	1.26
Clr/CCr	11	8	0.546	0.575	1.540	1.788	0.585	0.626	1.139	1.400
weight (kg)	11	8	60	55	96	85	69	63	78	77
SA (m2)	11	8	1.68	1.62	2.20	2.00	1.82	1.73	2.00	1.92

Appendix E16 Fit v Frail Elderly Males Matched for CCr - Mann-Whitney Test (contd)

	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	78.0	79.0	78.0	76.5	6.4	5.9	
mobility score	1.0	3.0	1.1	3.4	0.3	0.5	*
urine volume (ml)	1364	1628	1401	1915	385	1000	
UCr (mg in 24h)	1337	1228	1369	1332	299	630	
[UCr] (mg/100ml)	101.9	98.5	105.3	84.2	44.6	42.3	
[SCr] (mg/100ml)	1.100	0.965	1.106	1.249	0.239	0.617	
CCr (ml/min)	87.2	80.4	87.6	75.8	15.6	22.5	
CCr/SA (ml/min/1.73m2)	81.2	74.4	78.9	72.0	12.0	21.1	
dose (ug)	125.0	125.0	164.8	148.4	70.0	66.3	
%Du	46.72	59.63	44.98	55.33	12.39	22.31	
UDIG (ug in 24h)	62.0	64.8	69.3	77.4	24.8	41.7	
[UDIG] (ug/100ml)	4.23	4.05	5.24	5.61	2.21	4.44	
[SDIG] (ng/ml)	0.520	0.715	0.638	0.774	0.320	0.458	
Clt (ml/min)	118.7	107.8	143.6	106.0	77.4	46.5	
Clt/SA (ml/min/1.73m2)	115.2	101.4	128.8	101.0	67.9	45.4	
Clt/kg (ml/min/kg)	1.633	1.547	1.907	1.572	1.031	0.757	
Clr (ml/min)	87.9	60.9	86.9	84.4	33.0	53.2	
Clr/SA (ml/min/1.73m2)	80.6	58.9	78.3	78.5	29.8	45.2	
Clr/kg (ml/min/kg)	1.187	0.980	1.160	1.185	0.474	0.609	
Clnr (ml/min)	30.6	4.7	50.6	22.5	47.0	48.4	
Clnr/kg (ml/min/kg)	0.414	0.075	0.674	0.400	0.624	0.784	
Clr/CCr	1.056	1.043	0.978	1.068	0.322	0.437	
weight (kg)	75.0	66.0	75.6	68.8	11.5	9.6	
SA (m2)	1.910	1.820	1.918	1.821	0.168	0.123	

* $p<0.001$

Appendix F1

INFORMATION SHEET FOR PARACETAMOL STUDY

Dr. Parker is carrying out a project with the St. Marks Road GP's surgery, and the Research Institute for the Care of the Elderly, St. Martins Hospital, looking at the way in which paracetamol tablets are handled by your body.

These instructions should be followed carefully during the period of the study. If you are not sure what to do at any time then let someone know. Phone numbers are given at the bottom of this sheet. If you change your mind and do not wish to help, please also phone these numbers.

1) On the morning of the study Dr. Parker will call, and ask you to empty your bladder. You will then have a small blood sample taken. Following this, you will be required to take the two paracetamol tablets provided.

2) Each and every time you pass urine after taking the tablets, please collect it, in a separate container provided. Write on the container your name, and the time at which the urine was collected. ALL urine should be collected, even at night time. If you should happen to run out of containers in which to collect urine, a clean jam jar, or similar, will do. If you need to have your bowels open, empty your bladder first.

3) Exactly 24 hours later from when you took the paracetamol tablets, collect a final urine specimen, and save it.

4) Dr. Parker will need to take a further 5 blood samples during the day of the study (during the day time). Each time he needs a sample he will call at your house.

5) Dr. Parker will ask you at what time you got up, and went to bed on the study day, and how far you walked.

6) If you usually take tablets containing paracetamol, please do not take any from bedtime of the day before the study, and do not take any further paracetamol during the study until the blood sampling is complete.

7) If you are taking any other tablets you may take these as usual, but let Dr. Parker know.

Very many thanks.

IN CASE OF QUERY PLEASE DO NOT HESITATE TO CONTACT

SUE ELLMERS (STUDY CO-ORDINATOR) AT WORK ON BATH 835866 OR

AT HOME ON BATH 834963

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Appendix F2

STUDY TO MEASURE PARACETAMOL METABOLISM IN THE ELDERLY

Name : . . . . . Study No. . . . . .

Address : . . . . . Study Date. . . . . .

. . . . . Mobility Score. . . . .

. . . . . Height/cm. . . . . .

Tel. No.: . . . . . Weight/kg. . . . . .

D.O.B. : . . . . . Surface area/m^2. . . . .

Age : . . . . . .

G.P. : . . . . . .

Diagnoses : . . . . . .

. . . . . .

. . . . . .

. . . . . .

. . . . . .

Continent or catheterised:

Drugs : . . . . . .

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Intended Time for Par. to be taken :

Time Paracetamol taken : Dose Paracetamol taken :

		INTENDED	ACTUAL
Blood Samples :	0h	. . . . .	. . . . .
	3h	. . . . .	. . . . .
	4h	. . . . .	. . . . .
	6h	. . . . .	. . . . .
	9h	. . . . .	. . . . .
	12h	. . . . .	. . . . .

Time 24h urine colln started : . . . . . (discard urine)

Urine samples :	Time	Vol/ml	Combined
	1)	. . . . .	. . . . .
	2)	. . . . .	. . . . .
	3)	. . . . .	. . . . .
alcohol	4)	. . . . .	. . . . .
	5)	. . . . .	. . . . .
	6)	. . . . .	. . . . .
tobacco	7)	. . . . .	. . . . .
	8)	. . . . .	. . . . .
	9)	. . . . .	. . . . .
	10)	. . . . .	. . . . .
	11)	. . . . .	. . . . .
	12)	. . . . .	. . . . .

TOTAL URINE VOL. . . . . IN. . . . . hrs

Comments :

Appendix F3 Demographic Details of Subjects Taking Part in PARACETAMOL Study

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F01GPP	F / 78	59/153	Y / 2	no	no	palpitations dry skin, cramp	propranolol,Betnovate cream quinine sulphate
F02GPP	F / 88	50/160	Y / 1	no	3/wk	hypertension resected NG colon	Navidrex K, nitrazepam calcium, cod liver oil Monastry Herbs, yeast
F03GPP	F / 79	42/155	Y / 1	3/day	no	blind, MI 88, CHD thyroidectomy	frusemide paracetamol
F04GPP	F / 81	57/165	Y / 1	no	occ.	hypothyroid	thyroxine, cinnarizine
M05GPP	M / 82	75/175	Y / 2	no	occ.	recent UTI asthma	prednisolone, paracetamol terbutaline & budesonide inh.
F06GPP	F / 82	45/139	Y / 2	no	no	RA, hiatus hernia hypertension	Indocid,cimetidine, Gaviscon, Tenoret 50
F07GPP	F / 84	60/150	Y / 2	no	occ.	bilateral THR hypertension	astemizole, paracetamol Gaviscon, vits B & C
F08GPP	F / 74	50/148	Y / 1	no	occ.	minor skin lesion	Yeastvite
M09GPP	M / 75	76/160	Y / 1	no	occ.	macular degen.	salbutamol inh., Benylin
F10GPP	F / 80	51/152	Y / 1	no	occ.	red eyes, deaf	chloramphenicol eye ointment
M11GPP	M / 80	76/147	Y / 1	no	no	gout, hiatus hernia	oxytetracycline, Gaviscon
F12GPP	F / 74	67/164	Y / 1	no	no	blind R eye glaucoma, OA	hydrocortisone,pilocarpine & Propine eye drops
F13GPP	F / 74	66/161	Y / 1	>20/day	7/wk	Ca L breast mastectomy	tamoxifen, nitrazepam co-proxamol
M14P	M / 80	84/183	N / 4	no	14/wk	OA, leg ulcers # tib & fib	indomethacin
F15GPP	F / 67	87/153	Y / 1	no	1/wk	hiatus hernia URTI, phlebitis	Burinex K, ranitidine Gaviscon, co-proxamol
M16GPP	M / 73	56/158	Y / 1	1/4 oz	occ.	polymyalgia	naproxen, paracetamol
F17GPP	F / 78	75/148	Y / 2	no	no	OA	none
M18GPP	M / 69	69/169	Y / 1	pipe	no	migraine, partial thyroidectomy	paracetamol
F19GPP	F / 67	62/166	Y / 2	no	no	COAD night cramps	aminophylline SR,salbutamol & beclomethasone inh.,
M20P	M / 81	81/165	N / 3	no	no	R THR, knee pain	naproxen,paracetamol,senna
F21P	F / 93	38/145	N / 4	no	no	depression falls & # petit mal epilepsy	amitriptylline, lofepramine, KCl naproxen, paracetamol

Appendix F3 Demographic Details of Subjects Taking Part in PARACETAMOL Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F22P	F / 84	42/148	N / 3	no	no	AF, glaucoma R THR, blind R eye	digoxin, pilocarpine ED Asilone, Milpar
M23P	M / 88	55/165	N / 3	no	no	? Ca prostate IHD	cimetidine, Milpar paracetamol, multivitamins
M24P	M / 73	61/173	N / 3	5cigars	4/wk	hiatus hernia, TURP	terodiline, temazepam
M25P	M / 66	89/179	Y / 3	10/day	no	? normal pressure hydrocephalus, IHD	Milpar, paracetamol
F26P	F / 82	72/154	N / 4	no	no	osteoporosis # L & R femur, leg ulcer & cellulitis	ranitidine, diclofenac morphine sulphate, senna
F27P	F / 84	46/147	N / 3	no	no	cataracts, OA gastric erosions	Maxitrol ED
F28P	F / 80	67/156	N / 3	10/day	no	# L Colles haematuria	amitripylline, co-proxamol
M29P	M / 72	57/173	N / 4	no	no	CVA x 3 L. hemiparesis	enalapril,Milpar,frusemide hydralazine,metoprolol
F30GPP	F / 68	47/162	Y / 2	no	occ.	emphysema	salbutamol&beclomethasone inh., oxygen
M31GPP	M / 68	84/172	Y / 1	no	1/wk	OA, indigestion	Betnovate, mag.trisil.mix
M32P	M / 97	40/170	N / 5	no	no	bronchitis, R THR cataracts, deafness	temazepam, senna hydroxycobalamine
M33P	M / 76	74/169	N / 4	no	no	CVA x 2, TURP COAD, THR x 2	ranitidine, Neonaclex K prochlorperazine, diazepam aspirin, glycerine supps.
M34P	M / 86	46/164	N / 3	no	no	renal failure	folic acid
M35P	M / 71	54/159	Y / 3	10/day	occ.	Ca lung anaemia	ibuprofen,morphine sulphate prednisolone,lactulose
M36P	M / 73	50/156	Y / 3	no	?	gastric erosions iron deficiency anaemia, R THR ex-alcoholic	Praxilene, ranitidine multivitamins, senna paracetamol, FeSO4
F37P	F / 78	79/160	N / 4	no	no	epilepsy, UTIs CVA, L hemiplegia	phenytoin, temazepam paracetamol
F38P	F / 89	52/158	N / 5	no	no	URTI, Parkinsons cholecystectomy catheterised	co-benodopa, selegilene ibuprofen, lactulose co-amilofruse
F39P	F / 82	94/158	N / 4	no	2/wk	Ca R breast ? lung mets, partial thyroidectomy	co-amilofruse, GTN tamoxifen, naproxen

Appendix F3 Demographic Details of Subjects Taking Part in PARACETAMOL Study (contd)

subject	sex/age	wt/ht	fit/MSc	smoker	EtOH	diagnoses	drugs
F40P	F / 82	58/150	N / 4	no	no	cataracts depression	temazepam ispagula husk
F41P	F / 80	62/162	N / 4	no	no	psoriasis UTI dyspnoea	dithranol 2%, paracetamol senna, chlormethiazole Milpar, trimethoprim
F42GPP	F / 94	50/137	N / 3	no	no	arthritis	senna
F43P	F / 90	39/152	N / 3	no	no	IDDM, leg ulcer UTI, subarachnoid haemorrhage 1973	paracetamol sol. insulin
F44P	F / 89	70/150	N / 3	no	no	cholecystectomy 1974, URTI	bendrofluazide,ampicillin lofepramine,paracetamol
M45GPP	M / 64	54/173	N / 4	no	5/wk	MND	none
F46GPP	F / 86	68/158	N / 3	20/day	no	OA, seronegative arthropathy cholecystectomy colonic resection	prednisolone,Neonaclex K salbutamol,fenopren triazolam,lactulose paracetamol
F47GPP	F / 64	53/160	Y / 1	no	occ.	lumpectomy	tamoxifen
F48GPP	F / 94	36/150	Y / 2	no	occ.	kyphosis, eczema polymyalgia	Betnovate cream calcium, lactulose
F49GPP	F / 90	92/149	Y / 1	no	7/wk	bilat. mastectomy phlebitis	co-amilofruse co-proxamol
M50P	M / 83	--/175	N / 5	no	no	MND pressure sore	vit. C, aspirin, Milpar paracetamol, Fybogel
F51P	F / 93	40/140	N / 4	no	no	hypothyroid, OA depression	thyroxine, paracetamol lofepramine
F52P	F / 86	42/150	N / 4	no	no	infected leg ulcer	Milpar, paracetamol
M53GPP	M / 81	74/183	N / 4	no	?	CVAs, leg ulcer TURP, orchidectomy ex-alcoholic	paracetamol
F54GPP	F / 87	67/152	Y / 1	no	no	OA, cholecystectomy quiescent lymphoma	ranitidine, temazepam Gaviscon, paracetamol
M55GPP	M / 89	69/169	Y / 1	no	occ.	cholecystectomy	paracetamol
M56GPP	M / 87	60/173	Y / 1	no	7/wk	TUR, RIH repair hypertension	atenolol, ibuprofen co-amilozide

F = female
M = male
wt = weight (kg)
ht = height (cm)

ft = fit (Yes or No)
MSc = mobility score
EtOH = alcohol consumption in approx. units per week
occ. indicates irregular consumption of small amounts

Appendix F4 PARACETAMOL Kinetics in Fit and Frail Elderly People

subject start	T.int hours	U vol ml	UPAR mg	total UPAR mg	0-7h total UPAR mg/min	0-15h total UPAR mg/min	serum sample	T.int hours	[SPAR] mg/dl	kel	t1/2 hours	AUC	Cl l/h	Cl l/h/kg
F01GPP	7.00	236	10.1	368.7	0.878	0.888	1030	0.00	0.000	0.242	2.859	81.98	12.20	0.207
@1030	6.33	284	17.5	341.6			1320	2.83	1.540					
	10.25	880	26.4	183.8			1442	4.20	1.068					
	23.58	1400	14.0	894.1			1640	6.17	0.594					
							1930	9.00	0.293					
							2230	12.00	0.160					
F02GPP	7.00	140	10.2	572.4	1.363	0.966	1050	1.83	1.913	0.264	2.624	82.07	12.18	0.244
@0900	8.00	384	7.9	297.3			1255	3.92	1.006					
	8.50	334	6.4	91.8			1500	6.00	0.457					
	23.50	858	24.5	961.5			1810	9.17	0.177					
							2100	12.00	0.121					
F03GPP	8.00	182	10.4	436.6	0.910	0.734	0900	0.00	0.004	0.234	2.959	188.17	5.31	0.126
@0900	8.75	405	0.0	301.5			1200	3.00	6.578					
	7.25	420	0.0	63.8			1307	4.12	1.110					
	24.00	1007	10.4	801.9			1500	6.00	0.655					
							1755	8.92	0.339					
							2120	12.33	0.159					
F04GPP	5.50	370	29.5	414.6	1.256	0.990	0955	0.00	0.000	0.318	2.180	53.47	18.70	0.328
@1000	8.00	444	0.0	387.2			1257	2.95	1.051					
	10.75	820	0.0	196.2			1400	4.00	0.833					
	24.25	1634	29.5	998.0			1613	6.22	0.428					
							1845	8.75	0.194					
							2145	11.75	0.071					
F06GPP	6.00	154	7.3	153.1	0.425	0.555	0850	0.00	0.009	0.239	2.899	100.31	9.97	0.222
@0855	6.75	390	13.0	271.3			1200	3.08	1.763					
	11.17	350	10.1	175.7			1250	3.92	1.457					
	23.92	894	30.3	600.1			1500	6.08	0.804					
							1755	9.00	0.380					
							2103	12.13	0.206					
F07GPP	6.66	50	2.9	107.9	0.270	0.340	0925	0.00	0.017	0.291	2.383	64.69	15.46	0.258
@0930	6.17	110	5.9	153.9			1225	2.92	1.310					
	11.17	263	5.0	74.7			1332	4.03	0.977					
	24.00	423	13.8	336.5			1522	5.87	0.511					
							1830	9.00	0.204					
							2130	12.00	0.095					
F08GPP	7.75	355	15.6	215.9	0.464	0.406	0915	0.00	0.001	0.332	2.085	50.21	19.92	0.398
@0915	6.33	345	4.4	127.0			1218	3.05	1.011					
	9.92	740	5.4	62.0			1321	4.10	0.808					
	24.00	1440	25.4	404.9			1525	6.17	0.389					
							1819	9.07	0.158					
							2122	12.12	0.055					
F10GPP	5.50	184	20.5	208.5	0.632	1.232	1003	0.00	0.002	0.235	2.952	72.32	13.83	0.271
@1008	7.00	525	19.2	715.5			1311	3.05	1.186					
	11.75	675	0.0	73.2			1418	4.17	0.975					
	24.25	1384	39.7	997.2			1608	6.00	0.616					
							1915	9.12	0.315					
							2140	11.53	0.169					

Appendix F4 PARACETAMOL Kinetics in Fit and Frail Elderly People (contd)

subject start	T.int hours	U vol ml	UPAR mg	total UPAR mg	0-7h total UPAR mg/min	0-15h total UPAR mg/min	serum sample	T.int hours	[SPAR] mg/dl	kel	t1/2 hours	AUC	Cl l/h	Cl l/h/kg
F12GPP	7.42	338	0.0	640.3	1.438	0.924	0825	0.00	0.000	0.354	1.960	43.02	23.25	0.347
@0835	6.33	230	0.0	121.9			1146	3.18	0.877					
	9.50	532	0.0	91.2			1239	4.07	0.700					
	23.25	1100	0.0	853.5			1435	6.00	0.382					
							1728	8.88	0.142					
							2033	11.97	0.043					
F13GPP	6.08	260	11.3	637.0	1.746	1.048	0845	0.00	0.073	0.278	2.490	25.86	38.67	0.586
@0850	8.42	659	16.3	274.4			1200	3.17	0.450					
	9.50	855	13.3	77.8			1250	4.00	0.379					
	24.00	1774	40.9	989.1			1445	5.92	0.201					
							1737	8.78	0.099					
							2050	12.00	0.039					
F15GPP	5.67	108	6.1	284.8	0.837	0.659	1040	0.00	0.000	0.205	3.380	78.59	12.72	0.146
@1050	6.58	181	7.9	199.7			1408	3.30	1.192					
	12.25	830	0.0	158.3			1507	4.28	0.976					
	24.50	1119	14.0	642.8			1709	6.32	0.624					
							2002	9.20	0.392					
							2225	11.58	0.209					
F17GPP	7.50	688	1.9	483.3	1.074	0.749	0810	0.00	0.024	0.265	2.613	59.35	16.85	0.225
@0815	7.50	830	5.4	190.7			1115	3.00	1.040					
	8.42	890	7.4	50.0			1223	4.13	0.867					
	23.42	2408	14.7	724.0			1422	6.12	0.485					
							1714	8.98	0.223					
							2014	11.98	0.108					
F19GPP	5.83	99	8.0	181.7	0.519	0.573	0905	0.00	0.000	0.254	2.725	133.05	7.52	0.121
@0915	7.50	279	8.2	276.3			1220	3.08	2.648					
							1315	4.00	2.077					
	13.33	378	16.2	458.0			1535	6.33	0.783					
							1818	9.05	0.454					
							2115	12.00	0.253					
F21P							0923	0.00	0.187	0.243	2.849	107.51	9.30	0.245
@0930							1209	2.65	1.975					
							1326	3.93	1.482					
							1526	5.93	0.812					
							1820	8.83	0.410					
							2049	11.32	0.242					
F22P	6.58	62	8.2	183.6	0.464	0.481	1000	0.00	0.000	0.249	2.788	99.36	10.06	0.240
@1003	3.50	32	4.2	107.4			1306	3.05	1.776					
	14.00	414	33.9	299.8			1403	4.00	1.452					
	24.08	508	46.3	590.9			1540	5.62	0.896					
							1829	8.43	0.444					
							2100	10.95	0.256					
F26P	6.42	612	24.9	370.8	0.962	0.791	0950	0.00	0.041	0.289	2.397	50.13	19.95	0.281
@0952	7.08	538	13.2	269.7			1255	3.05	0.936					
	8.00	1118	21.0	173.9			1348	3.93	0.722					
	21.50	2268	59.1	814.4			1550	5.97	0.426					
							1853	9.02	0.219					
							2135	11.72	0.071					

Appendix F4 PARACETAMOL Kinetics in Fit and Frail Elderly People (contd)

subject start	T.int hours	U vol ml	UPAR mg	total UPAR mg	0-7h total UPAR mg/min	0-15h total UPAR mg/min	serum sample	T.int hours	[SPAR] mg/dl	kel	t1/2 hours	AUC	Cl l/h	Cl l/h/kg
F27P	6.92	160	10.0	299.9	0.722	0.392	0840	0.00	0.000	0.233	2.977	82.83	12.07	0.268
@0845	7.17	208	10.8	31.3			1156	3.18	1.385					
	10.08	430	1.6	44.0			1245	4.00	1.166					
	24.17	798	22.5	375.1			1530	6.75	0.600					
							1749	9.07	0.322					
							2115	12.50	0.164					
F28P	7.17	214	13.3	708.4	1.647		0759	0.00	0.314	0.220	3.146	61.45	16.27	0.243
@0800							1050	2.83	1.158					
							1201	4.02	0.722					
	7.17	214	13.3	708.4			1403	6.05	0.372					
							1714	9.23	0.166					
							2003	12.05	0.125					
F30GPP	7.13	426	27.2	446.0	1.043	0.756	0721	0.00	0.054	0.314	2.204	48.97	20.42	0.434
@0728	6.62	638	12.5	177.8			1025	2.95	0.983					
	10.08	910	17.4	52.5			1131	4.05	0.743					
	23.83	1974	57.1	676.3			1339	6.18	0.375					
							1609	8.68	0.161					
							1821	10.80	0.091					
F37P	7.42	442	21.5	469.3	1.054	1.062	0920	0.00	0.000	0.374	1.853			
@0923	2.08	156	5.4	135.8			1237	3.23	0.390					
	14.67	777	17.8	153.9			1350	4.45	0.284					
	24.17	1375	44.8	758.9			1615	6.87	0.119					
							1822	9.98	0.036					
							2125	12.03	0.000					
F38P	7.00	160	0.0	154.7	0.368	0.363	0840	0.00	0.164	0.114	6.089	86.67	11.54	0.222
@0847	5.25	102	0.0	112.4			1205	3.30	0.967					
	11.92	320	0.0	275.4			1335	4.80	0.652					
	24.17	582	0.0	542.5			1545	6.97	0.470					
							1810	9.38	0.396					
							2110	12.38	0.266					
F39P	4.17	305	5.9	132.5	0.529	0.627	1205	3.02	0.809	0.228	3.038	47.92	20.87	0.222
@0904	9.33	654	13.3	375.5			1307	4.05	0.648					
	10.67	840	8.6	127.9			1600	6.93	0.318					
	24.17	1799	27.8	635.8			1800	8.93	0.187					
							2103	11.98	0.108					
F40P	6.08	45	5.1	110.5	0.303	0.650	1215	3.03	1.602	0.284	2.440	81.26	12.31	0.199
@0913	2.67	216	11.4	230.6			1319	4.10	1.566					
	15.08	850	15.6	361.3			1607	6.90	0.939					
	23.83	1111	32.1	702.4			1807	8.90	0.625					
							2110	11.95	0.315					
F41P	6.00	102	1.1	113.3	0.315		1205	3.03	1.602	0.284	2.440	81.26	12.31	0.199
@0903							1304	4.02	1.208					
							1515	6.20	0.594					
	6.00	102	1.1	113.3			1810	9.12	0.307					
							2105	12.03	0.117					

Appendix F4 PARACETAMOL Kinetics in Fit and Frail Elderly People (contd)

subject start	T.int hours	U vol ml	UPAR mg	total UPAR mg	0-7h total UPAR mg/min	0-15h total UPAR mg/min	serum sample	T.int hours	[SPAR] mg/dl	kel	t1/2 hours	AUC	Cl l/h	Cl l/h/kg
F42GPP	6.47	52	2.9	121.5	0.313	0.678	1130	2.90	1.406	0.233	2.976	134.57	7.43	0.149
@0836	6.50	198	8.1	406.3			1234	3.97	2.193					
	9.60	110	3.9	60.4			1436	6.00	1.330					
	22.57	360	14.9	588.2			1743	9.12	0.635					
							2031	11.92	0.345					
F43P	4.30	28	1.0	36.2	0.140		1024	3.08	1.353	0.290	2.393	65.12	15.36	0.394
@0719							1128	4.15	0.903					
							1313	5.90	0.520					
	4.30	28	1.0	36.2			1618	8.98	0.259					
							1910	11.85	0.091					
F44P	5.33	91	5.1	223.9	0.700		1218	3.02	1.227	0.263	2.636	62.50	16.00	0.229
@0917							1317	4.00	0.871					
							1515	5.97	0.502					
	5.33	91	5.1	223.9			1817	9.00	0.232					
							2115	11.97	0.106					
F46GPP	6.17	170	8.4	391.6	1.058	1.007	1222	2.98	0.880	0.315	2.198	39.12	25.56	0.376
@0923	4.75	404	6.4	267.9			1317	3.90	0.611					
							1535	6.20	0.266					
	10.92	574	14.8	659.5			1833	9.17	0.102					
							2114	11.85	0.050					
F47GPP	6.00	235	20.3	541.0	1.503	0.994	1245	2.92	0.805	0.255	2.718	39.27	25.46	0.480
@0950	7.25	502	16.9	249.0			1354	4.07	0.525					
	10.75	564	9.6	70.3			1554	6.07	0.283					
	24.00	1301	46.8	860.3			1855	9.08	0.128					
							2154	12.07	0.068					
F48GPP	6.25	94	10.4	153.5	0.409	0.678	1246	2.93	2.374	0.267	2.591	127.93	7.82	0.217
@0950	7.83	334	21.1	419.5			1348	3.97	1.848					
	8.17	220	7.9	120.6			1549	5.98	1.080					
	22.25	648	39.4	693.6			1856	9.10	0.450					
							2155	12.08	0.214					
F49GPP	3.73	284	14.4	203.4	0.909	0.778	1216	2.98	0.951	0.265	2.612	48.19	20.75	0.226
@0917	8.00	158	23.7	344.3			1314	3.95	0.708					
	11.75	426	0.0	132.7			1519	6.05	0.373					
	23.48	868	38.1	680.4			1819	9.03	0.153					
							2116	11.98	0.086					
F51P	6.67	70	9.2	184.8	0.462		1202	2.93	1.760	0.235	2.947	97.39	10.27	0.257
@0906	15.00	750	38.0	399.3			1257	3.85	1.380					
							1505	5.98	0.763					
	21.67	820	47.2	584.1			1830	9.40	0.328					
							2058	11.87	0.212					
F52P	2.92	160	6.8	112.9	0.644	0.660	1155	2.77	1.386	0.213	3.260	76.38	13.09	0.312
@0909	8.42	186	10.2	336.3			1252	3.72	0.998					
	6.35	322	5.7	189.2			1458	5.82	0.588					
	17.69	668	22.8	638.4			1822	9.22	0.286					
							2050	11.68	0.183					

Appendix F4 PARACETAMOL Kinetics in Fit and Frail Elderly People (contd)

Subject start	T.int hours	U vol ml	UPAR mg	total UPAR mg	0-7h total UPAR mg/min	0-15h total UPAR mg/min	serum sample	T.int hours	[SPAR] mg/dl	kel	t1/2 hours	AUC	Cl l/h	Cl l/h/kg
F54GPP							1124	2.73	1.049	0.281	2.463	50.80	19.69	0.294
@0840							1238	3.97	0.728					
							1439	5.98	0.372					
							1740	9.00	0.168					
							2037	11.95	0.075					
M05GPP	6.00	116	9.7	265.3	0.737	1.393	1000	0.00	0.000	0.335	2.070	53.21	18.79	0.251
@1000	7.50	297	12.0	369.8			1253	2.88	0.989					
	10.75	780	7.0	165.9			1400	4.00	0.817					
	24.25	1193	28.6	801.0			1615	6.25	0.464					
							1855	8.92	0.205					
							2140	11.67	0.062					
M09GPP	7.50	545	60.9	723.1	1.607	1.270	0910	0.00	0.001	0.221	3.131	61.82	16.18	0.213
@0915	6.50	705	19.7	343.7			1215	3.00	0.883					
	10.00	770	1.7	125.4			1320	4.08	0.803					
	24.00	2020	82.3	1192.1			1522	6.12	0.595					
							1815	9.00	0.259					
							2120	12.08	0.147					
M11GPP	7.50	252	9.4	373.1	0.829	0.740	0827	0.00	0.000	0.297	2.330	46.93	21.31	0.280
@0835	6.25	265	0.0	237.7			1150	3.25	0.862					
	10.17	499	0.0	164.6			1242	4.12	0.715					
	23.92	1016	9.4	775.4			1437	6.03	0.427					
							1730	8.92	0.188					
							2036	12.02	0.068					
M14P	4.22	240	10.2	164.7	0.651	0.592	0950	0.00	0.099	0.180	3.861	57.06	17.53	0.209
@0952	8.77	330	6.8	296.7			1258	3.10	0.836					
	10.43	515	0.0	187.6			1351	3.98	0.678					
	23.42	1085	17.0	649.0			1600	6.13	0.410					
							1902	9.17	0.234					
							2210	12.30	0.151					
M16GPP	4.00	140	8.3	112.4	0.468		1041	0.00	0.088	0.178	3.602	64.72	15.45	0.276
@1050							1406	3.27	0.963					
							1504	4.23	0.763					
	4.00	140	8.3	112.4			1704	6.23	0.440					
							2000	9.17	0.219					
							2222	11.53	0.220					
M18GPP	7.92	499	13.5	600.1	1.263	0.833	0825	0.00	0.024	0.241	2.874	43.13	23.19	0.336
@0830	7.53	780	6.9	172.4			1126	2.93	0.739					
	8.25	563	0.0	41.3			1235	4.08	0.590					
	23.70	1842	20.4	813.8			1425	5.92	0.359					
							1726	8.93	0.172					
							2030	12.00	0.087					
M20P	6.57	76	8.0	97.4	0.247	0.416	0853	0.00	0.002	0.259	2.679	59.62	16.77	0.210
@0855	5.66	170	13.2	207.7			1200	3.08	1.194					
	11.77	776	17.5	275.0			1258	4.05	0.883					
	24.00	1022	38.8	580.1			1509	6.23	0.398					
							1810	9.25	0.184					
							2040	11.75	0.119					

Appendix F4 PARACETAMOL Kinetics in Fit and Frail Elderly People (contd)

Subject start	T.int hours	U vol ml	UPAR mg	total UPAR mg	0-7h total UPAR mg/min	0-15h total UPAR mg/min	serum sample	T.int hours	[SPAR] mg/dl	kel	t1/2 hours	AUC	Cl l/h	Cl l/h/kg
M23P	6.67	60	2.8	149.9	0.375	1.062	0830	0.00	0.086	0.207	3.343	69.88	14.13	0.260
@0830	5.42	235	11.5	620.3			1130	3.00	1.160					
	12.08	422	18.8				1230	4.00	0.926					
	24.17	717	33.1	770.2			1430	6.00	0.500					
							1740	9.17	0.260					
							2034	12.07	0.170					
M24P	7.50	418	9.5	282.6	0.628	0.646	0910	0.00	0.058	0.186	3.734	76.52	13.07	0.214
@0913	5.00	445	5.7	202.3			1232	3.33	1.077					
	11.75	735	17.1	443.9			1318	4.08	0.989					
	24.25	1598	32.3	928.8			1514	6.02	0.597					
							1804	8.85	0.353					
							2114	12.02	0.222					
M25P							0900	0.00	0.000	0.352	1.971	37.86	26.41	0.297
@0930							1223	2.88	0.912					
							1326	3.93	0.657					
							1519	5.82	0.238					
							1812	8.70	0.098					
							2122	11.87	0.037					
M29P	6.25	234	5.6	84.6	0.226	0.445	1025	0.00	0.000	0.172	4.025	40.99	24.40	0.436
@1030	5.08	235	6.0	217.8			1344	3.23	0.647					
							1441	4.18	0.465					
	11.33	469	11.5	302.4			1638	6.13	0.285					
							1940	9.17	0.155					
							2158	11.47	0.136					
M31GPP	7.33	626	33.7	506.2	1.151	0.765	0717	0.00	0.000	0.245	2.834	33.37	29.97	0.357
@0725	8.42	964	18.6	217.2			1023	2.97	0.604					
	8.25	740	0.0	164.3			1133	4.13	0.431					
	24.00	2330	52.3	887.7			1340	6.25	0.268					
							1610	8.75	0.147					
							1813	10.80	0.084					
M32P	21.33	360	26.7	544.8		0.426	0927	0.00	0.000	0.153	4.533	112.79	8.87	0.227
@0930							1240	3.17	1.411					
							1337	4.12	1.214					
	21.33	360	26.7	544.8			1530	6.00	0.875					
							1830	9.00	0.519					
							2132	12.03	0.367					
M33P	7.50	164	18.3	229.3	0.510	0.536	0907	0.00	0.000	0.257	2.699	67.08	14.91	0.201
@0908	6.00	260	19.3	204.7			1204	2.93	1.289					
	11.17	698	27.4	184.6			1307	3.98	0.958					
	24.67	1122	65.0	618.6			1510	6.03	0.518					
							1810	9.03	0.232					
							2114	12.10	0.119					
M34P	6.83	152	16.5	32.4	0.079	0.144	0900	0.00	0.153	0.095	7.332	173.07	5.78	0.126
@0907	3.84	216	18.9	59.8			1208	3.02	1.447					
	12.08	896	66.1	197.0			1308	4.02	1.336					
	22.75	1264	101.5	289.2			1510	6.05	0.987					
							1812	9.08	0.733					
							2110	12.05	0.620					

Appendix F4 PARACETAMOL Kinetics in Fit and Frail Elderly People (contd)

Subject start	T.int hours	U vol ml	UPAR mg	total UPAR mg	0-7h total UPAR mg/min	0-15h total UPAR mg/min	serum sample	T.int hours	[SPAR] mg/dl	kel	t1/2 hours	AUC	Cl l/h	Cl l/h/kg
M35P	6.70	238	15.9	322.1	0.801	0.753	0848	0.00	0.000	0.261	2.655	51.58	19.39	0.359
@0850	5.55	668	23.9	231.4			1217	3.45	0.908					
	10.25	1205	26.3	147.0			1322	4.53	0.675					
	22.50	2111	66.0	700.5			1526	6.60	0.416					
							1828	9.63	0.179					
							2105	12.25	0.092					
M36P	6.33	44	5.2	210.0	0.553	0.739	0900	0.00	0.062	0.308	2.247	46.22	21.64	0.433
@0905	5.92	70	8.0	333.1			1210	3.08	0.971					
	11.75	481	18.7	203.8			1315	4.17	0.647					
	24.00	595	31.9	746.9			1510	6.08	0.338					
							1820	9.25	0.139					
							2115	12.17	0.053					
M45GPP	5.42	465	31.4	354.5	1.090	0.708	1032	3.03	1.475	0.246	2.812	82.61	12.11	0.224
@0730	7.10	126	4.7	177.7			1133	4.05	1.179					
	12.23	390	13.1	227.4			1335	6.08	0.643					
	24.75	981	49.2	759.6			1633	9.05	0.327					
							1943	12.22	0.153					
M50P	6.20	190	7.5	116.4	0.313	0.469	1217	3.07	1.028	0.238	2.910	54.46	18.36	*
@0913	5.55	324	26.6	214.0			1328	4.25	0.686					
	12.33	778	0.0	266.1			1519	6.10	0.386					
	24.08	1292	34.1	596.5			2055	11.70	0.112					
M53GPP							1138	3.13	0.241	0.157	4.417	80.05	12.49	0.169
@0830							1232	4.03	0.939					
							1441	6.18	0.908					
							1739	9.15	0.494					
							2135	13.08	0.247					
M55GPP	5.67	214	11.2	226.4	0.666	0.716	1133	3.00	0.913	0.195	3.556	57.67	17.34	0.251
@0833	8.58	642	10.1	385.7			1232	3.98	0.720					
	9.25	746	0.0	138.9			1429	5.93	0.457					
	23.50	1602	21.4	751.1			1717	8.73	0.263					
							2030	11.95	0.151					
M56GPP	3.67	106	2.7	54.8	0.249	0.552	1151	2.98	0.932	0.204	3.395	56.13	17.82	0.297
@0853	10.67	546	2.2	420.3			1251	3.98	0.700					
	9.75	1082	5.8	282.1			1445	5.87	0.452					
	24.09	1734	10.7	757.2			1730	8.62	0.266					
							2049	11.93	0.136					

Appendix F5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in PARACETAMOL Study

subject/ start	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F01GPP	1.46	1630	7.00	134.1	56.83	236	22		79		
0930		2250	6.33	186.6	65.71	284	34		121		
		0905	10.25	250.2	28.43	880	28		100		
Totals			23.58	570.9	40.78	1400		28		1.60	30
F02GPP	1.80	1800	7.00	223.6	159.87	140	30		91		
1100		0200	8.00	350.8	91.35	384	41		124		
		1030	8.50	343.9	102.97	334	37		112		
Totals			23.50	918.3	107.03	858		33		1.50	38
F03GPP	1.58	1700	8.00	177.7	97.66	182	23		85		
0900		0145	8.75	285.5	70.49	405	34		126		
		0900	7.25	161.9	38.55	420	24		89		
Totals			24.00	625.1	62.08	1007		27		1.35	35
F04GPP	1.17	1530	5.50	204.9	55.37	370	53		110		
1000		2330	8.00	244.8	55.14	444	44		92		
		1015	10.75	377.0	45.98	820	50		104		
Totals			24.25	826.7	50.60	1634		48		1.63	51
F06GPP	2.11	1455	6.00	84.8	55.07	154	11		65		
0855		2140	6.75	191.0	48.98	390	22		129		
		0850	11.17	227.4	64.85	350	16		94		
Total			23.92	503.2	56.29	894		17		1.34	21
F07GPP	1.27	1610	6.66	38.7	77.35	50	8		53		
0930		2220	6.17	90.6	82.40	110	19		127		
		0930	11.17	148.9	56.63	263	17		113		
Totals			24.00	278.2	65.77	423		15		1.60	16
F08GPP	0.82	1700	7.75	252.2	71.04	355	66		110		
0915		2320	6.33	174.6	50.61	345	56		93		
		0915	9.92	284.6	38.47	740	58		97		
Totals			24.00	711.4	49.40	1440		60		1.45	72
F10GPP	1.15	1530	5.50	81.36	44.22	184	21		54		
1000		2230	7.00	400.6	76.31	525	83		213		
		1015	11.75	174.6	25.86	675	22		56		
Totals			24.25	656.6	47.44	1384		39		1.48	46
F12GPP	0.91	1555	7.42	293.22	86.75	338	72		97		
0830		2215	6.33	256.73	111.62	230	74		100		
		0745	9.50	390.82	73.46	532	75		101		
Totals			23.25	940.8	85.52	1100		74		1.76	73
F13GPP	1.03	1450	6.08	257.3	98.96	260	68		86		
0845		2315	8.42	471.6	71.56	659	91		115		
		0845	9.50	446.6	52.23	855	76		96		
Totals			24.00	1175.5	66.26	1774		79		1.74	79
F15GPP	0.92	1630	5.67	268.9	249.02	108	86		110		
1050		2305	6.58	302.3	167.04	181	83		106		
		1120	12.25	482.8	58.17	830	71		91		
Totals			24.50	1054.0	94.19	1119		78		1.96	69

Appendix F5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in PARACETAMOL Study (contd)

subject/ start	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F17GPP	0.92	1545	7.50	308.4	44.82	688	74		93		
0815		2315	7.50	373.7	45.03	830	90		113		
		0740	8.42	356.5	40.06	890	77		96		
Totals			23.42	1038.6	43.13	2408		80		1.79	78
F19GPP	1.53	1500	5.83	122.9	124.20	99	23		55		
0910		2230	7.50	256.1	91.79	279	37		88		
		0910	10.67	542.4	226.01	240	55		131		
Totals			24.00	921.4	149.10	618		42		1.46	50
F22P	2.14	1630	6.58	105.0	169.34	62	12		92		
0955		2000	3.50	52.9	165.44	32	12		92		
		1000	14.00	240.3	58.04	414	13		100		
Totals			24.08	398.2	78.38	508		13		1.33	17
F26P	0.90	1525	6.42	171.0	27.94	612	49		111		
0900		2230	7.08	167.4	31.11	538	44		100		
		0630	8.00	168.8	15.10	1118	39		89		
Totals			21.50	507.2	22.36	2268		44		1.77	43
F27P	0.69	1535	6.92	169.4	105.87	160	59		190		
0840		2245	7.17	46.7	22.46	208	16		52		
		0850	10.08	98.4	22.88	430	24		77		
Totals			24.17	314.5	39.41	798		31		1.37	40
F30GPP	1.00	1438	7.13	180.0	43.66	426	43		91		
0730		2115	6.62	217.6	34.10	638	55		117		
		0720	10.08	266.2	29.25	910	44		94		
Totals			23.83	669.8	33.93	1974		47		1.46	56
F37P	1.00	1640	7.42	306.0	69.23	442	69		130		
0915		1845	2.08	90.1	57.76	156	72		138		
		0925	14.67	366.4	47.16	777	42		79		
Totals			24.17	762.5	55.46	1375		53		1.90	48
F38P	0.89	1550	7.00	110.2	68.86	160	29		104		
0850		2105	5.25	41.6	40.74	102	15		54		
		0900	11.92	212.6	66.43	320	33		118		
Totals			24.17	364.4	62.61	582		28		1.52	32
F39P	1.53	1300	4.17	158.0	51.81	305	41		87		
0850		2220	9.33	385.8	58.99	654	45		96		
		0900	10.67	491.1	58.47	840	50		106		
Totals			24.17	1034.9	57.53	1799		47		2.07	39
F40P	1.25	1520	6.08	63.0	139.88	45	14		47		
0915		1800	2.67	159.2	73.70	216	79		263		
		0905	15.08	321.7	37.85	850	28		93		
Totals			23.83	543.9	48.96	1111		30		1.58	33
F41P	1.39	1445	24.00	459.4	31.90	1440	23	23		1.69	23
0845											

Appendix F5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in PARACETAMOL Study (contd)

subject/ start	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
F42GPP	0.59	1500	6.47	129.1	248.25	52	56		74		
0832		2130	6.50	359.9	181.77	198	156		205		
		0706	9.60	117.1	106.47	110	34		45		
Totals			22.57	606.1	168.40	360		76		1.41	93
F44P	0.88	1330	11.00	384.5	86.80	443	66		114		
0230		2025	6.92	256.8	92.71	277	70		121		
		0330	7.08	128.4	46.20	278	34		59		
Totals			25.00	769.7	77.13	998		58		1.74	58
F46GPP	1.01	1530	6.17	292.0	171.75	170	78		132		
0920		2015	4.75	207.0	51.24	404	72		122		
		0830	12.25	335.4	145.85	230	45		76		
Totals			23.17	834.4	103.78	804		59		1.75	59
F47GPP	0.69	1545	6.00	258.6	110.06	235	104		101		
0945		2300	7.25	327.2	65.17	502	109		106		
		0945	10.75	439.2	77.87	564	99		96		
Totals			24.00	1025.0	78.79	1301		103		1.55	115
F48GPP	0.78	1600	6.25	101.4	107.84	94	35		85		
0945		2350	7.83	202.8	60.72	334	55		134		
		0800	8.17	125.6	57.07	220	33		80		
Totals			22.25	429.8	66.32	648		41		1.23	58
F49GPP	0.82	1300	3.73	101.7	35.82	284	55		86		
0916		2100	8.00	240.1	151.95	158	61		95		
		0845	11.75	396.1	92.98	426	69		108		
Totals			23.48	737.9	85.01	868		64		1.99	56
F51P	1.16	1545	6.67	70.3	100.46	70	15		68		
0905		0645	15.00	266.8	35.57	750	26		118		
Totals			21.67	337.1	41.11	820		22		1.26	31
F52P	1.19	1200	2.92	123.0	76.87	160	59		148		
0909		2025	8.42	200.6	107.87	186	33		83		
		0246	6.35	175.6	52.72	322	37		93		
Totals			17.69	499.2	74.72	668		40		1.34	52
F54GPP	1.70	1505	6.50	305.1	44.61	684	46		112		
0835		2200	6.92	278.7	46.76	596	39		95		
		0910	11.17	439.5	62.78	700	39		95		
Totals				1023.3	51.68	1980		41		1.71	41
F21P	2.80									1.25	
F28P	2.06									1.73	
F43P	0.50									1.29	
M05GPP	1.68	1600	6.00	230.1	198.37	116	38		80		
1000		2330	7.50	387.3	130.42	297	51		104		
		1015	10.75	571.6	73.28	780	53		108		
Totals			24.25	1189.0	99.67	1193		49		1.92	44

Appendix F5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in PARACETAMOL Study (contd)

subject/ start	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M09GPP	1.29	1645	7.50	509.7	93.53	545	88		117		
0830		2315	6.50	375.4	53.25	705	75		100		
		0915	10.00	506.4	65.77	770	65		87		
Totals			24.00	1391.5	68.89	2020		75		1.86	70
M11GPP	1.30	1600	7.50	385.7	153.05	252	66		97		
0830		2215	6.25	363.0	136.98	265	74		109		
		0825	10.17	526.0	105.42	499	66		97		
Totals			23.92	1274.7	125.46	1016		68		1.80	66
M14P	0.69	1403	4.22	227.1	94.64	240	130		124		
0950		2249	8.77	379.1	114.88	330	104		99		
		0915	10.43	412.7	80.15	515	96		91		
Totals			23.42	1018.9	93.91	1085		105		2.06	88
M16GPP	0.81	2300	5.50	287.4	105.28	273	108		140		
1730		0845	9.75	278.5	57.79	482	59		77		
		1750	9.08	344.1	35.99	956	78		101		
Totals			24.33	910.0	53.18	1711		77		1.58	84
M18GPP	1.61	1625	7.92	526.4	105.49	499	69		117		
0830		2357	7.53	392.3	50.30	780	54		92		
		0812	8.25	421.8	74.92	563	53		90		
Totals			23.70	1340.5	72.77	1842		59		1.81	56
M20P	3.78	1524	6.57	158.8	208.93	76	11		69		
0850		2104	5.66	227.7	133.93	170	18		113		
		0850	11.77	459.2	59.17	776	17		106		
Totals			24.00	845.7	82.75	1022		16		1.94	14
M23P	2.38	1500	6.67	56.9	94.84	60	6		30		
0820		2025	5.42	260.6	110.89	235	34		170		
		0830	12.08	362.0	85.78	422	21		105		
Totals			24.17	679.5	94.77	717		20		1.60	21
M24P	2.24	1630	7.50	1188.1	284.22	418	118		155		
0900		2130	5.00	574.7	129.14	445	86		113		
		0915	11.75	716.8	97.52	735	45		59		
Totals			24.25	2479.6	155.17	1598		76		1.72	77
M29P	1.47	2025	10.08	63.6	10.51	605	7		44		
1030		1025	14.00	266.2	43.00	619	22		138		
Totals			24.08	329.8	26.94	1224		16		1.64	16
M31GPP	1.11	1435	7.33	470.0	75.08	626	96		107		
0715		2300	8.42	525.3		964	94		104		
		0715	8.25	449.7		740	82		91		
Totals			24.00	1445.0		2330		90		2.02	77
M32P	1.65	0640	21.33	354.4	98.44	360	17	17		1.36	21
0920											

Appendix F5 SCr, UCr & CCr, calculated over 8 & 24h periods, for Subjects in PARACETAMOL Study (contd)

subject/ start	[SCr] mg/dl	urine colln	T.int hours	total mg UCr	[UCr] mg/dl	urine vol/ml	CCr ml/min	24h CCr ml/min	% 24h CCr	SA m2	24h CCr 1.73m2
M33P	0.94	1530	7.50	304.5	185.67	164	72		90		
0800		2130	6.00	282.5	108.64	260	83		104		
		0840	11.17	523.3	74.97	698	83		104		
Totals			24.67	1111.1	99.03	1122		80		1.88	73
M34P	3.07	1550	6.83	76.1	50.06	152	6		60		
0900		1940	3.84	83.9	38.83	216	12		120		
		0745	12.08	243.0	27.12	896	11		110		
Totals			22.75	403.0	31.88	1264		10		1.45	11
M35P	0.90	1542	6.70	196.3	82.47	238	54		95		
0900		2115	5.55	201.3	30.14	668	67		118		
		0730	10.25	300.9	24.97	1205	54		95		
Totals			22.50	698.5	33.09	2111		57		1.56	64
M36P	1.10	1520	6.33	136.2	309.51	44	33		75		
0900		2115	5.92	178.5	254.93	70	46		105		
		0900	11.75	375.6	78.09	481	48		109		
Totals			24.00	690.3	116.01	595		44		1.48	51
M45GPP	0.65	1250	5.42	122.7	26.39	465	58		95		
0725		1956	7.10	96.4	76.47	126	35		57		
		0810	12.23	367.9	94.33	390	77		126		
Totals			24.75	587.0	59.84	981		61		1.61	65
M50P	2.36	1527	6.20	92.6	48.73	190	11		85	*	
0915		2100	5.55	107.9	33.31	324	14		108		
		0920	12.33	242.7	31.19	778	14		108		
Totals			24.08	443.2	34.30	1292		13			
M55GPP	1.87	1410	5.67	258.2	120.67	214	41		91		
0830		2245	8.58	479.8	74.73	642	50		111		
		0800	9.25	456.7	61.22	746	44		98		
Totals			23.50	1194.7	74.58	1602		45		1.81	43
M56GPP	1.89	1140	3.67	100.8	95.11	106	24		65		
0800		2220	10.67	420.2	76.96	546	35		95		
		0805	9.75	494.6	45.71	1082	45		122		
Totals			24.09	1015.6	58.57	1734		37		1.70	38
M25P	1.87									2.12	
M53GPP	3.23									1.94	

Appendix F6 Results from All Subjects Taking PARACETAMOL

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	55	64	97	74	87	81	80.3	8.3
t1/2 (h)	55	1.960	7.332	2.463	3.260	2.834	3.004	0.928
Cl (l/h)	55	5.31	38.67	12.11	19.95	16.00	16.263	6.421
Cl (l/h/kg)	54	0.121	0.586	0.214	0.316	0.248	0.2689	0.0947
[SPAR] @ 3h (mg/dl)	51	0.450	6.578	0.913	1.411	1.051	1.304	0.872
[SPAR] @ 4h (mg/dl)	53	0.379	2.193	0.704	1.138	0.871	0.959	0.385
urine vol. (ml)	50	360	2408	816	1653	1120.5	1236.1	76.4
free UPAR (mg)	51	0.0	101.5	14.0	40.9	27.8	30.51	21.34
UPARG+S (mg)	43	187.7	1109.8	562.3	793.4	670.4	680.8	184.8
total UPAR (mg)	43	289.2	1192.1	596.5	814.4	702.4	715.1	185.3
UCr (mg in 24h)	50	315	2480	502	1028	724.7	805.5	55.6
[UCr] (mg/100ml)	50	22.4	168.4	47.5	94.3	66.29	72.75	4.89
SCr (mg/100ml)	55	0.50	3.78	0.90	1.70	1.19	1.382	0.656
CCr (ml/min)	50	10	105	28	70	45	48.0	25.0
CCr/SA (ml/min/1.73m2)	49	11	115	33	70	51	50.7	23.7
weight (kg)	54	36	40	50	72	60	60.8	15.1
SA (m2)	54	1.23	2.12	1.46	1.80	1.62	1.640	0.235

Appendix F7 Spearmans Coefficient of Rank Correlation for All Subjects Taking PARACETAMOL

	age years	M.Sc	t1/2 hours	Cl l/h	Cl l/h/kg	urine vol ml	free UPAR mg	UPAR G+S mg	total UPAR mg	UCr mg	SCr mg/dl	CCr ml/min	CCr 1.73m2	weight kg
n	55	55	55	55	54	50	51	43	43	50	55	50	49	54
M.Sc	0.282													
t1/2	0.133	0.255												
Cl	0.394 ***	0.309	0.402 ***											
Cl/kg	0.169	0.249	0.377 ***	0.731 ****										
U vol	0.467 ***	0.358	0.047	0.516 ****	0.275									
free UPAR	0.179	0.051	0.034	0.039	0.004	0.249								
UPAR G+S	0.378	0.572 ****	0.154	0.375	0.258	0.462 ***	0.050							
total UPAR	0.415 ***	0.536 ****	0.172	0.381	0.276	0.496 ***	0.140	0.992 ****						
UCr	0.391 ***	0.533 ****	0.149	0.507 ****	0.101	0.494 ****	0.046	0.551 ****	0.534 ****					
SCr	0.135	0.063	0.200	0.137	0.230	0.040	0.124	0.028	0.028	0.039				
CCr	0.450 ***	0.368 ***	0.159	0.453 ***	0.178	0.377 ***	0.005	0.434 ***	0.426 ***	0.730 ****	0.599 ****			
CCr/SA	0.416 ***	0.321	0.157	0.385 ***	0.211	0.304	0.004	0.384 ***	0.371 ***	0.596 ****	0.650 ****	0.963 ****		
weight	0.327	0.197	0.105	0.548 ****	0.114	0.443 ***	0.047	0.284	0.277	0.720 ****	0.034	0.520 ****	0.315	
SA	0.334	0.161	0.055	0.598 ****	0.043	0.481 ****	0.014	0.326	0.321	0.723 ****	0.053	0.528 ****	0.315	0.973 ****

*** p<0.01 **** p<0.001

Appendix F8 Results from Female Subjects Taking PARACETAMOL

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	33	64	94	78	89	82	81.8	8.0
t1/2 (h)	33	1.960	6.089	2.418	2.968	2.718	2.795	0.693
Cl (l/h)	33	5.31	38.67	10.17	19.94	13.83	15.41	6.86
Cl (l/h/kg)	33	0.121	0.586	0.219	0.320	0.244	0.2695	0.1040
[SPAR] @ 3h (mg/dl)	31	0.450	6.578	0.967	1.760	1.192	1.490	1.066
[SPAR] @ 4h (mg/dl)	32	0.379	2.193	0.724	1.337	0.976	1.053	0.435
urine vol. (ml)	30	360	2408	766	1488	1054	1166	552
free UPAR (mg)	26	0.0	59.1	14.8	42.2	28.7	29.95	16.38
UPARG+S (mg)	25	322.7	968.5	557.2	826.8	642.3	673.4	184.1
total UPAR (mg)	25	336.5	998.0	589.5	856.9	680.3	703.4	188.1
UCr (mg in 24h)	30	315	1176	489	926	647.4	695.8	252.2
[UCr] (mg/100ml)	30	22.4	168.4	42.6	85.1	62.3	69.9	6.4
[SCr] (mg/100ml)	33	0.50	2.80	0.89	1.50	1.06	1.201	0.496
CCr (ml/min)	30	13	103	28	61	42	46.2	22.6
CCr/SA (ml/min/1.73m2)	30	16	115	33	62	48	50.1	23.0
weight (kg)	33	36	94	45	67	57	57.7	15.2
SA (m2)	33	1.23	2.07	1.36	1.74	1.55	1.567	0.222

Appendix F9 Spearmans Coefficient of Rank Correlation for Female Subjects Taking PARACETAMOL

	age years	M.Sc	t1/2 hours	Cl l/h	Cl l/h/kg	urine vol. ml	free UPAR mg	UPAR G+S mg	total UPAR mg	UCr mg	SCr mg/dl	CCr ml/min	CCr 1.73m2	weight kg
n	33	33	33	33	33	30	26	25	25	30	33	30	30	33
M.Sc	0.489 ***													
t1/2	0.130	0.361												
Cl	0.301	0.305	0.542 ***											
Cl/kg	0.083	0.185	0.566 ***	0.718 ****										
U vol	0.544 ***	0.298	0.318	0.563 ***	0.303									
free UPAR	0.152	0.019	0.166	0.188	0.273	0.352								
UPAR G+S	0.352	0.525 ***	0.237	0.288	0.148	0.515	0.214							
total UPAR	0.370	0.511 ***	0.252	0.306	0.180	0.548 ***	0.308	0.993 ****						
UCr	0.443	0.513 ***	0.299	0.559 ***	0.183	0.460	0.169	0.461	0.433					
SCr	0.066	0.061	0.154	0.298	0.277	0.050	0.070	0.101	0.137	0.002				
CCr	0.272	0.390	0.274	0.600 ***	0.335	0.369	0.058	0.372	0.334	0.736 ****	0.612 ****			
CCr/SA	0.193	0.379	0.261	0.470	0.370	0.217	0.079	0.333	0.290	0.583 ***	0.685 ****	0.947 ****		
weight	0.323	0.181	0.118	0.582 ****	0.076	0.452	0.108	0.259	0.239	0.656 ****	0.083	0.518 ***	0.271	
SA	0.329	0.220	0.166	0.662 ****	0.021	0.527 ***	0.118	0.354	0.332	0.661 ****	0.111	0.538 ***	0.289	0.974 ****

*** p<0.01 **** p<0.001

Appendix F10 Results from Male Subjects Taking PARACETAMOL

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	22	64	97	72	84	78	77.9	8.4
t1/2 (h)	22	1.971	7.332	2.673	3.766	3.021	3.319	1.145
Cl (l/h)	22	5.78	29.97	14.00	21.39	17.44	17.55	5.61
Cl (l/h/kg)	22	0.126	0.436	0.211	0.317	0.251	0.2679	0.0805
[SPAR] @ 3h (mg/dl)	20	0.640	1.475	0.867	1.186	0.967	1.017	0.249
[SPAR] @ 4h (mg/dl)	21	0.431	1.336	0.668	0.949	0.763	0.814	0.234
urine vol. (ml)	20	360	2330	1018	1728	1244	1341	519
free UPAR (mg)	20	8.3	101.5	17.9	51.5	32.1	37.03	25.46
UPARG+S (mg)	18	187.7	1109.8	560.2	777.6	722.4	691.2	190.5
total UPAR (mg)	18	289.2	1192.1	613.1	804.2	754.1	731.2	185.5
UCr (mg in 24h)	20	330	2480	610	1255	963	970	504
[UCr] (mg/100ml)	20	26.9	155.2	54.5	98.9	73.7	77.1	33.9
SCr (mg/100ml)	22	0.65	3.78	1.01	1.98	1.63	1.653	0.777
CCr (ml/min)	20	10	105	18	76	53	50.8	28.7
CCr/SA (ml/min/1.73m2)	19	11	88	21	73	56	51.5	25.3
weight (kg)	21	39	89	55	76	69	65.8	13.9
SA (m2)	21	1.36	2.12	1.59	1.93	1.80	1.755	0.210

Appendix F11 Spearmans Coefficient of Rank Correlation for Male Subjects Taking PARACETAMOL

	age years	M.Sc	t1/2 hours	Cl l/h	Cl l/h/kg	urine vol ml	free UPAR mg	UPAR G+S mg	total UPAR mg	UCr mg	[SCr] mg/dl	CCr ml/min	CCr 1.73m2	weight kg
n	22	22	22	22	21	20	20	18	18	20	22	20	19	21
M.Sc	0.098													
t1/2	0.409	0.252												
Cl	0.457	0.349	0.560											
Cl/kg	0.500	0.396	0.552	0.861 ****										
U vol	0.313	0.558	0.056	0.355	0.267									
free UPAR	0.130	0.217	0.164	0.295	0.296	0.137								
UPAR G+S	0.372	0.718 ***	0.242	0.445	0.405	0.477	0.211							
total UPAR	0.456	0.651 ***	0.265	0.361	0.356	0.411	0.135	0.981 ****						
UCr	0.241	0.673 ***	0.288	0.274	0.021	0.495	0.047	0.803 ****	0.776 ***					
SCr	0.565 ***	0.004	0.185	0.175	0.278	0.049	0.095	0.108	0.183	0.117				
CCr	0.518	0.296	0.188	0.123	0.004	0.278	0.074	0.501	0.558	0.709 ***	0.691 ***			
CCr/SA	0.523	0.173	0.157	0.133	0.007	0.304	0.078	0.463	0.507	0.624 ***	0.688 ***	0.981 ****		
weight	0.201	0.284	0.354	0.437	0.020	0.270	0.065	0.399	0.385	0.716 ***	0.006	0.544	0.439	
SA	0.198	0.119	0.288	0.358	0.057	0.385	0.006	0.337	0.337	0.661 ***	0.029	0.515	0.389	0.953 ****

*** p<0.01 **** p<0.001

Appendix F12 Female v Male Elderly Subjects Taking PARACETAMOL - Mann-Whitney Test

	female n	male n	female median	male median	female mean	male mean	female s.d.	male s.d.	p
age (years)	33	22	82.0	78.0	81.8	77.9	8.0	8.4	
mobility score	33	22	2.0	3.0	2.4	2.7	1.3	1.4	
t1/2 (hours)	33	22	2.718	3.021	2.795	3.319	0.693	1.145	*
Cl (l/h)	33	22	13.83	17.44	15.41	17.55	6.86	5.61	
Cl (l/h/kg)	33	21	0.270	0.251	0.244	0.268	0.104	0.081	
[SPAR] @ 3h (mg/dl)	31	20	1.192	0.967	1.490	1.017	1.066	0.249	**
[SPAR] @ 4h (mg/dl)	32	21	0.976	0.763	1.053	0.814	0.435	0.234	*
urine volume (ml)	30	20	1054	1244	1166	1341	552	519	
free UPAR (mg)	31	20	25.37	32.08	26.30	37.03	17.37	25.46	
UPARG+S (mg)	25	18	642.3	722.4	673.4	691.2	184.1	190.5	
total UPAR (mg)	25	18	680.3	754.1	703.4	731.2	188.1	185.5	
UCr (mg in 24h)	30	20	647	963	696	970	252	504	*
[UCr] (mg/100ml)	30	20	62.34	73.67	69.87	77.06	35.22	33.94	
[SCr] (mg/100ml)	33	22	1.060	1.630	1.201	1.653	0.496	0.777	**
CCr (ml/min)	30	20	41.5	53.0	46.2	50.8	22.6	28.7	
CCr/SA (ml/min/1.73m2)	30	19	48.0	56.0	50.1	51.5	23.0	25.3	
weight (kg)	33	21	57.0	69.0	57.7	65.8	15.2	13.9	*
SA (m2)	33	21	1.550	1.800	1.567	1.755	0.222	0.210	***

* $p<0.05$ ** $p<0.02$ *** $p<0.005$

Appendix F13 Fit v Frail Elderly Subjects Taking PARACETAMOL - Mann-Whitney Test

	fit n	frail n	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	29	26	78	83	77.3	83.5	8.2	7.3	***
mobility score	29	26	1	4	1.5	3.7	0.7	0.7	****
t1/2 (hours)	29	26	2.624	2.977	2.668	3.379	0.457	1.162	****
Cl (l/h)	29	26	18.70	13.07	18.210	13.980	6.990	5.070	**
Cl (l/h/kg)	29	25	0.276	0.228	0.292	0.243	0.105	0.076	*
[SPAR] @ 3h (mg/dl)	27	24	0.983	1.258	1.318	1.289	1.155	0.379	
[SPAR] @ 4h (mg/dl)	28	25	0.783	0.958	0.880	1.047	0.376	0.382	
urine volume (ml)	28	22	1392	1010	1392	1037	551	465	**
free UPAR (mg)	27	24	28.64	27.74	30.59	30.42	19.81	23.38	
UPARG+S (mg)	25	18	766.0	567.8	749.5	585.5	176.8	153.7	****
total UPAR (mg)	25	18	775.4	607.5	781.6	622.7	183.5	147.5	****
total UPAR (mg) 0 - 7h	25	18	0.837	0.487	0.889	0.575	0.419	0.369	***
total UPAR (mg) 0 - 15h	25	18	0.755	0.610	0.809	0.608	0.249	0.239	***
UCr (mg in 24h)	28	22	919.8	526.0	905.6	678.0	290.3	471.0	****
[UCr] (mg/100ml)	28	22	66.29	68.67	73.36	71.97	31.32	39.01	
[SCr] (mg/100ml)	29	26	1.17	1.22	1.279	1.497	0.409	0.846	
CCr (ml/min)	28	22	49	31	54.2	40.2	22.2	26.7	*
CCr/SA (ml/min/1.73m2)	28	21	56	39	56.3	43.1	21.2	25.2	
weight (kg)	29	25	60	56	62.9	58.4	14.6	15.7	
SA (m2)	29	25	1.63	1.61	1.668	1.612	0.222	0.250	

* p<0.05 ** p<0.02 *** p<0.01 **** p<0.003

Appendix F14 Fit v Frail Elderly Females Taking PARACETAMOL - Mann-Whitney Test

	fit n	frail n	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	18	15	79	84	78.3	86.1	8.5	4.9	***
mobility score	18	15	1	4	1.4	3.7	0.5	0.7	****
t1/2 (hours)	18	15	2.613	2.947	2.594	3.035	0.354	0.913	*
Cl (l/h)	18	15	16.16	12.31	16.71	13.84	7.90	5.18	
Cl (l/h/kg)	18	15	0.251	0.243	0.285	0.251	0.125	0.071	
[SPAR] @ 3h (mg/dl)	17	14	1.051	1.369	1.577	1.384	1.402	0.424	
[SPAR] @ 4h (mg/dl)	18	14	0.921	1.082	0.988	1.137	0.428	0.446	
urine volume (ml)	18	12	1210	1013	1268	812	535	564	
free UPAR (mg)	17	14	29.1	22.9	29.10	22.91	16.25	18.67	
UPARG+S (mg)	19	9	750.4	573.3	727.2	577.7	198.0	110.2	**
total UPAR (mg)	16	9	762.9	590.9	757.1	608.0	201.4	118.8	**
FPAR/PARG+S	16	9	0.043	0.052	0.043	0.052	0.024	0.029	
total UPAR (mg) 0 - 7h	16	9	0.909	0.529	0.922	0.645	0.435	0.396	
total UPAR (mg) 0 - 15h	16	9	0.756	0.655	0.781	0.671	0.236	0.234	
UCr (mg in 24h)	18	12	782.3	503.2	789.2	555.8	231.9	221.1	**
[UCr] (mg/100ml)	18	12	64.2	60.1	71.7	67.2	33.3	39.3	
[SCr] (mg/100ml)	18	15	1.09	1.06	1.203	1.199	0.406	0.601	
CCr (ml/min)	18	12	45	36	50.9	39.3	24.5	18.4	
CCr/SA (ml/min/1.73m2)	18	12	54	40	54.7	43.3	24.1	20.3	
weight (kg)	18	15	58	52	59.2	55.9	14.8	16.0	
SA (m2)	18	15	1.58	1.52	1.589	0.154	0.207	0.244	

* p<0.05 ** p<0.03 *** p<0.01 **** p<0.001

Appendix F15 Fit v Frail Elderly Males Taking PARACETAMOL - Mann-Whitney Test

	fit n	frail n	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	11	11	73	81	75.7	80.1	7.7	8.9	
mobility score	11	11	1	4	1.6	3.8	0.9	0.8	****
t1/2 (hours)	11	11	2.834	3.734	2.788	3.850	0.587	1.337	**
Cl (l/h)	11	11	19.39	14.31	20.68	14.31	4.46	4.97	***
Cl (l/h/kg)	11	10	0.297	0.212	0.305	0.228	0.062	0.082	**
[SPAR] @ 3h (mg/dl)	10	10	0.913	1.177	0.877	1.156	0.119	0.270	**
[SPAR] @ 4h (mg/dl)	10	11	0.708	0.939	0.684	0.932	0.113	0.256	**
urine volume (ml)	10	10	1722	1104	1615	1066	535	338	*
free UPAR (mg)	10	10	25.0	33.6	33.12	40.93	25.54	26.12	
UPARG+S (mg)	9	9	766.0	562.3	789.2	593.2	132.6	194.7	**
total UPAR (mg)	9	9	775.4	618.6	825.1	637.4	147.3	177.7	*
FPAR/PARG+S	9	9	0.037	0.061	0.045	0.113	0.030	0.163	
total UPAR (mg) 0 - 7h	9	9	0.769	0.375	0.832	0.458	0.405	0.304	**
total UPAR (mg) 0 - 15h	9	9	0.753	0.503	0.862	0.544	0.278	0.239	***
UCr (mg in 24h)	10	10	1192	633	1115	825	275	643	*
[UCr] (mg/100ml)	10	10	70.8	88.3	76.4	77.7	28.9	39.9	
[SCr] (mg/100ml)	11	11	1.30	1.71	1.403	1.904	0.400	0.985	
CCr (ml/min)	10	10	58	19	60.1	41.4	17.1	35.4	
CCr/SA (ml/min/1.73m2)	10	9	60	21	59.3	42.9	15.4	31.9	
weight (kg)	11	10	69	59	68.9	62.3	12.6	15.0	
SA (m2)	11	10	1.81	1.68	1.787	1.720	0.196	0.229	

* p<0.05 ** p<0.02 *** p<0.006 **** p<0.001

Appendix F16 Results from Age-Sex Matched Group Taking PARACETAMOL

	n	min	max	Q1	Q3	median	mean	s.d.
age (years)	32	64	94	77	88	82	81.8	7.4
t1/2 (h)	32	1.971	7.332	2.405	3.393	2.905	3.104	1.102
Cl (l/h)	32	5.78	26.41	12.13	18.77	15.46	15.685	5.208
Cl (l/h/kg)	31	0.126	0.436	0.214	0.294	0.251	0.2583	0.0754
[SPAR] @ 3h (mg/dl)	30	0.647	2.374	0.927	1.361	1.050	1.186	0.388
[SPAR] @ 4h (mg/dl)	31	0.465	2.193	0.708	1.166	0.833	0.966	0.383
urine volume (ml)	29	360	2020	801	1519	1111.0	1135.8	449.8
free UPAR (mg)	26	0.0	101.5	16.5	38.4	29.9	31.88	22.86
UPARG+S (mg)	25	187.7	1109.8	566.1	769.2	670.3	677.7	208.4
total UPAR (mg)	25	289.2	1192.1	598.3	788.2	702.4	710.8	208.7
UCr (mg in 24h)	29	315	2480	458	1021	690.3	804.0	446.8
[UCr] (mg/100ml)	29	26.9	168.4	49.8	101.7	66.33	77.03	38.3
[SCr] (mg/100ml)	32	0.50	3.07	0.84	1.85	1.26	1.368	0.622
CCr (ml/min)	29	10	105	26	66	41	45.6	24.6
CCr/SA (ml/min/1.73m2)	28	11	93	32	66	45	48.4	22.9
weight (kg)	31	36	92	50	69	58	60.7	13.9
SA (m2)	31	1.23	2.12	1.48	1.80	1.63	1.647	0.219

Appendix F17 Spearmans Coefficient of Rank Correlation for Age-Sex Matched Subjects Taking PARACETAMOL

	age years	M.Sc	t1/2 hours	Cl l/h	Cl l/h/kg	urine vol ml	free UPAR mg	UPAR G+S mg	total UPAR mg	UCr mg	[SCr] mg/dl	CCr ml/min	CCr 1.73m2	weight kg
n	32	32	32	32	31	29	30	24	24	29	32	29	28	31
M.Sc	0.185													
t1/2	0.015	0.233												
Cl	0.285	0.238	0.426 ***											
Cl/kg	0.096	0.325	0.378	0.702 ****										
U vol	0.368	0.239	0.193	0.256	0.084									
free UPAR	0.195	0.007	0.057	0.273	0.423	0.083								
UPAR G+S	0.295	0.561 ***	0.201	0.312	0.329	0.520	0.095							
total UPAR	0.366	0.510	0.234	0.306	0.318	0.523	0.183	0.986 ****						
UCr	0.168	0.451	0.186	0.422	0.059	0.408	0.020	0.604 ***	0.604 ***					
[SCr]	0.068	0.132	0.110	0.135	0.019	0.402	0.066	0.210	0.197	0.144				
CCr	0.254	0.230	0.112	0.220	0.084	0.148	0.093	0.364	0.371	0.702 ****	0.523 ***			
CCr/SA	0.242	0.128	0.115	0.161	0.107	0.094	0.181	0.288	0.288	0.558 ***	0.569 ***	0.970 ****		
weight	0.298	0.147	0.165	0.638 ****	0.019	0.392	0.067	0.250	0.268	0.704 ****	0.155	0.515 ****	0.345	
SA	0.344	0.081	0.129	0.660 ****	0.018	0.414	0.046	0.284	0.314	0.718 ****	0.188	0.529 ****	0.345	0.975 ****

*** p<0.01 **** p<0.001

Appendix F18 Fit v Frail Age-Sex Matched Subjects Taking PARACETAMOL - Mann-Whitney Test

	fit n	frail n	fit median	frail median	fit mean	frail mean	fit s.d.	frail s.d.	p
age (years)	16	16	82	82	81.9	81.7	7.4	7.7	
mobility score	16	16	1	4	1.6	3.6	0.7	0.7	***
t1/2 (hours)	16	16	2.602	3.062	2.688	3.520	0.519	1.368	*
Cl (l/h)	16	16	17.08	14.29	17.08	14.29	4.68	5.47	
Cl (l/h/kg)	16	15	0.264	0.222	0.272	0.243	0.054	0.093	
[SPAR] @ 3h (mg/dl)	15	15	0.971	1.160	1.141	1.231	0.411	0.371	
[SPAR] @ 4h (mg/dl)	16	15	0.783	0.958	0.897	1.039	0.323	0.437	
urine volume (ml)	15	14	1193	1098	1237	1027	522	343	
free UPAR (mg)	14	16	29.5	32.1	30.73	33.03	18.59	27.20	
UPARG+S (mg)	13	11	746.5	602.5	760.9	587.5	202.5	181.6	**
total UPAR (mg)	13	11	757.2	633.8	791.6	623.3	213.1	171.5	*
total UPAR (mg) 0 - 7h	13	11	0.666	0.375	0.762	0.573	0.424	0.415	
total UPAR (mg) 0 - 15h	13	11	0.740	0.592	0.842	0.592	0.311	0.251	*
UCr (mg in 24h)	15	14	910	565	876	727	308	562	
[UCr] (mg/100ml)	15	14	68.9	61.2	78.9	75.1	31.2	45.8	
[SCr] (mg/100ml)	16	16	1.30	1.04	1.413	1.324	0.434	0.778	
CCr (ml/min)	15	14	44	31	46.2	44.9	18.5	30.5	
CCr/SA (ml/min/1.73m2)	15	13	46	40	48.2	48.5	17.6	28.6	
weight (kg)	16	15	60	56	63.1	58.1	15.7	11.8	
SA (m2)	16	15	1.67	1.61	1.672	1.620	0.240	0.200	

* $p<0.03$ ** $p<0.02$ *** $p<0.0001$